GENETIC ALTERATIONS RELATED TO FAMILIAL ALZHEIMER'S DISEASE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of U.S. Appl. No. 08/706,344, filed August 30, 1996, which claims benefit of the filing date of U.S. Appl. No. 60/003,054, filed August 31, 1995.

STATEMENT REGARDING FEDERALLY-SPONSORED RESEARCH AND DEVELOPMENT

[0002] Statement under MPEP 310. The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of contract No. (RO1) NS30428 awarded by National Institutes of Health.

BACKGROUND OF THE INVENTION

Field of the Invention

[0003] The present invention relates generally to Alzheimer's disease, and more specifically to methods and compositions for use in diagnosis and treatment of Alzheimer's Disease.

Background Art

[0004] Alzheimer's disease (AD) is a devastating neurodegenerative progressive disorder, which is the predominant cause of dementia in people over 65 years of age. The prevalence of AD is estimated to be as high as 18.7% among 75-84 year-olds and 47.2% among the ≥85 year age groups, affecting a significant portion of the population in most countries of the world.

[0005] Clinical symptoms of the disease typically begin with subtle short term memory problems. As the disease progresses, difficulty with memory, language, and orientation worsen to the point of interfering with the ability of the person to function independently. Other symptoms, which are variable, include myoclonus and seizures. Duration of AD from the first symptoms of memory loss until death is 10 years on average, but may range from 6-8 years to more than 20 years. AD always results in death, often from respiratory-related illness.

[0006] The pathology in AD is confined exclusively to the central nervous system (CNS). The AD brain is characterized by the presence of amyloid deposits and neurofibrillary tangles (NFT).

[0007] Amyloid deposits are found associated with the vascular system of the CNS and as focal deposits in the parenchyma. The major molecular component of an amyloid deposit is a highly hydrophobic peptide called the Aβ peptide. This peptide aggregates into filaments in an anti-β-pleated sheet structure resulting in the birefringent nature of the AD amyloid. Although Aβ is the major component of AD amyloid, other proteins have also found associated with the amyloid, *e.g.*, α-1-anti-chymotrypsin (Abraham, *et al.*, *Cell 52*:487-501 (1988)), cathepsin D (Cataldo *et al.*, *Brain Res. 513*:181-192 (1990)), non-amyloid component protein (Ueda *et al.*, *Proc. Natl. Acad. Sci. USA 90*:11282-11286 (1993)), apolipoprotein E (apoE) (Namba *et al.*, *Brain Res. 541*:163-166 (1991); Wisniewski & Frangione, *Neurosci. Lett. 135*:235-238 (1992); Strittmatter *et al.*, *Proc. Natl. Acad. Sci. USA 90*:1977-1981 (1993)), apolipoprotein J (Choi-Mura *et al.*, *Acta Neuropathol. 83*:260-264 (1992); McGeer *et al.*, *Brain Res. 579*:337-341 (1992)), heat shock protein 70 (Hamos *et al.*, *Neurology 41*:345-350 (1991)), complement components (McGeer & Rogers, *Neurology 43*:447-449 (1992)), α_2-macroglobin (Strauss *et al.*, *Lab. Invest. 66*:223-230 (1992)), interleukin-6 (Strauss *et al.*, *Lab. Invest. 66*:223-230 (1992)), proteoglycans (Snow *et al.*, *Lab. Invest. 58*:454-458 (1987)), and serum amyloid P (Coria *et al.*, *Lab. Invest. 58*:454-458 (1988)).

[0008] Plaques are often surrounded by astrocytes and activated microglial cells expressing immune-related proteins, such as the MHC class II glycoproteins HLA-

DR, HLA-DP, and HLA-DQ, as well as MHC class I glycoproteins, interleukin-2 (IL-2) receptors, and IL-1. Also surrounding many plaques are dystrophic neurites, which are nerve endings containing abnormal filamentous structures.

[0009] The characteristic Alzheimer's NFTs consist of abnormal filaments bundled together in neuronal cell bodies. "Ghost" NFTs are also observed in AD brains, which presumably mark the location of dead neurons. Other neuropathological features include granulovacular changes, neuronal loss, gliosis and the variable presence of Lewy bodies.

[0010] The destructive process of the disease is evident on a gross level in the AD brain to the extent that in late-stage AD, ventricular enlargement and shrinkage of the brain can be observed by magnetic resonance imaging. The cells remaining at autopsy, however, are grossly different from those of a normal brain, characterized by extensive gliosis and neuronal loss. Neurons which were possibly involved in initiating events, are absent; and other cell types, such as the activated microglial cells and astrocytes, have gene expression patterns not observed in the normal brain. Thus, the amyloid plaque structures and NFTs observed at autopsy are most likely the end-products of a lengthy disease process, far removed from the initiating events of AD.

[0011] Accordingly, attempts to use biochemical methods to identify key proteins and genes in the initiating steps of the disease are hampered by the fact that it is not possible to actually observe these critical initiating events. Rather, biochemical dissection of the AD brain at autopsy is akin to molecular archeology, attempting to reconstruct the pathogenic pathway by comparing the normal brain to the end-stage disease brain.

[0012] Substantial evidence has suggested that inherited genetic defects are involved in AD. Numerous kindreds have been described in the literature as having early-onset AD (defined as onset before age 65). Bird *et al.*, *Ann. Neurol. 23*:25-31 (1988); Bird *et al.*, *Ann. Neurol, 25*:12-25 (1989); Cook *et al.*, *Neurology 29*:1402-1412 (1979); Feldman *et al.*, *Neurology 13*:811-824 (1960); Goudsmit, *J. Neurol. Sci. 49*:79 (1981); Heston & White, *Behavior Genet. 8*:315-

331 (1978); Martin *et al.*, *Neurology 41*:62-68 (1991); Nee *et al.*, *Arch. Neurol. 40*:203-208 (1983); van Bogaeert *et al.*, *Mschr. Psych. Neurol. 102*:249-301 (1940); Wheelan, *Ann. Hum. Genet. 23*:300-309 (1959)). Families with multiple late-onset AD cases have also been described (Bird *et al.*, *Ann. Neurol.* (1989), *supra*; Heston & White, *Behavior Genet.* (1978), *supra*; Pericak-Vance *et al.*, *Exp. Neurol. 102*:271-279 (1988)). In addition, twin studies have documented that monozygotic twins are more concordant in their AD phenotype than dizygotic twins (Nee *et al.*, *Neurology 37*:359-363 (1987). Also, the families of concordant twins have more secondary cases of AD than families of discordant twins (Rapoport *et al.*, *Neurology 41*:1549-1553 (1991)).

[0013] Genetic dissection of AD has been complicated by the complexity and overall accuracy of its diagnosis. Because AD is relatively common in the elderly, clustering of cases in a family may occur by chance, representing possible confounding non-allelic genetic heterogeneity, or etiologic heterogeneity with genetic and non-genetic cases co-existing in the same kindred. In addition, the clinical diagnosis of AD is confounded with other dementing diseases common in the elderly.

[0014] Despite these problems, mutations in the amyloid precursor protein (APP) gene on chromosome 21 have been associated with early-onset (< 65 years) autosomal dominant AD (Goate *et al.*, *Nature 349*:704 (1991)). Moreover, mutations in two recently identified genes, S182 on chromosome 14 and STM-2 on chromosome 1, which encode presenilin 1 (PS1) and presenilin 2 (PS2), respectively, have also been associated with early-onset autosomal dominant AD (Schellenberg *et al.*, *Science 258*:668 (1992); Sherrington *et al.*, *Nature 375*:754-760 (1995); Levy-Lahad/Wasco *et al.*, *Science 269*:973-977 (1995)).

[0015] For late-onset AD, the APOE gene has been identified as a genetic modifying factor (Strittmatter *et al.*, *Proc. Natl. Acad. Sci. USA 90*:1977 (1993); Corder *et al.*, *Science 261*:921 (1993); Corder *et al.*, *Nat. Genet. 7*:180-184 (1994); Benjamin *et al.*, *Lancet 344*:473 (1994); Smith *et al.*, *Lancet 344*:473-474 (1994)).

[0016] However, the known genetic loci for AD do not account for all cases of AD. For example, in late-onset AD approximately half of AD cases do not have the APOE ϵ4 allele found in several other families with high incidence of AD, including the Volga German (VG) kindreds. Brousseau *et al.*, *Neurology 342* (1994); Kuusisto *et al.*, *Brit. Med. J. 309*:363 (1994); Tsai *et al.*, *Am. J. Hum. Genet. 54*:643 (1994); Liddel *et al.*, *J. Med. Genet. 31*:197 (1994); Cook *et al.*, *Neurology* (1979), *supra*; Bird *et al.*, *Ann. Neurol.* (1988), *supra*; Bird *et al.*, *Ann. Neurol. 25*:12 (1989). The known AD loci have been excluded as possible causes of the discrepancy. Schellenberg *et al.*, *Science* (1992), *supra*; Lannfelt *et al.*, *Nat. Genet. 4*:218-219 (1993)); van Duijn *et al.*, *Am. J Hum. Genet. 55*:714-727 (1994); Schellenberg *et al.*, *Science 241*:1507 (1988); Schellenberg *et al.*, *Am. J. Hum. Genet. 48*:563 (1991); Schellenberg *et al.*, *Am. J. Hum. Genet. 49*:511-517 (1991); Kamino *et al.*, *Am. J. Hum. Genet. 51*:998 (1992); Schellenberg *et al.*, *Am J. Hum. Genet. 53*:619 (1993); Schellenberg *et al.*, *Ann. Neurol. 31*:223 (1992); Yu *et al.*, *Am. Hum. Genet. 54*:631 (1994)). Thus, identification of new genes and of risk-modifying alterations of existing genes will add considerably to an understanding of the genetic determinants of AD, and enable biochemical and genetic approaches to the diagnosis and therapeutic treatment.

[0017] The present invention provides novel, previously unidentified and apparently pathogenic mutations of the chromosomal loci for familial AD (FAD), methods and compositions for diagnosis and treatment of AD, and other related advantages.

BRIEF SUMMARY OF THE INVENTION

[0018] Briefly stated, the present invention provides isolated nucleic acid molecules encoding a PS1 gene product. A representative nucleic acid molecule is provided in Fig. 2A-Fig. 2F, while in other embodiments, nucleic acid molecules are provided which encode a mutant PS1 gene product that increases the probability of Alzheimer's disease (in a statistically significant manner). One

representative illustration of such a mutant is an amino acid substitution at residue 263, wherein, for example, an arginine may be substituted for a cysteine (C263R) (SEQ ID NO:28). Another representative illustration of such a mutant is an amino acid substitution at residue 264, wherein, for example, a leucine may be substituted for a proline (P264L) (SEQ ID NO:30). A third representative illustration of such a mutant is an amino acid substitution at residue 269, wherein, for example, a histidine may be substituted for an arginine (R269H) (SEQ ID NO:32).

[0019] Other aspects of the present invention included isolated nucleic acid molecules, selected from the group consisting of: a) an isolated nucleic acid molecule as set forth in Fig. 2A-Fig. 2F, or complementary sequence thereof; b) an isolated nucleic acid molecule that specifically hybridizes to the nucleic acid molecule of (a) under conditions of high stringency; and c) an isolated nucleic acid that encodes a PS1 gene product. As utilized herein, it should be understood that a nucleic acid molecule hybridizes "specifically" to a PS1 gene (or related sequence) if it hybridizes detectably to such a sequence, but does not usually hybridize to the PS2 gene sequence under the same conditions. The invention also provides methods of obtaining said nucleic acid molecules, fragments thereof, or functional derivatives thereof.

[0020] The present invention also provides expression vectors comprising a promoter operably linked to one of the nucleic acid molecules described above. Within related aspects, viral vectors are provided that are capable of directing the expression of a nucleic acid molecule as described above. Also provided are host cells which carry the above-described vectors.

[0021] The present invention further provides isolated proteins comprising a PS1 gene product, as well as PS1 peptides of greater than 12, 13, or 20 amino acids. Within one embodiment, a protein is provided that has the amino acid sequence set forth in Fig. 2A-Fig. 2F. Within another embodiment, the protein is a mutant PS1 gene product that increases the probability of Alzheimer's disease. Such mutants include those with an amino acid substitution at residue 263 (*e.g.*, an

arginine:cysteine substitution), or at residue 264 (*e.g.*, a leucine:proline substitution), or at residue 269 (*e.g.*, a histidine:arginine substitution). In addition, PS1 peptides are provided which are composed of 13 to 20 amino acids derived or selected from the N-terminal, internal, or carboxyl-terminal hydrophilic regions.

[0022] Within yet another embodiment of the present invention, methods of treating or preventing Alzheimer's disease are provided, comprising the step of administering to a patient a vector containing or expressing a nucleic acid molecule, protein, or antibody specific for a PS1 protein as described above, thereby reducing the likelihood or delaying the onset of Alzheimer's disease in the patient. Within certain embodiments, the above methods may be accomplished by *in vivo* administration.

[0023] Also provided by the present invention are pharmaceutical compositions comprising a nucleic acid molecule, vector, host cell, protein, or antibody as described above, along with a pharmaceutically acceptable carrier or diluent.

[0024] In addition, the present invention provides antibodies which specifically bind to a PS1 protein, or to immunological equivalent, unique peptides derived from the N-terminal, internal, or carboxyl-terminal hydrophilic regions. As utilized herein, it should be understood that an antibody is specific for a PS1 protein if it binds detectably, and with a K_A of 10^{-7}M or less, but does not bind detectably (or with an affinity of greater than 10^{-7}M) to the PS2 protein. Also provided are hybridomas which are capable of producing such antibodies. The antibodies of the present invention include monoclonal and polyclonal antibodies, as well fragments of these antibodies, and humanized forms.

[0025] The present invention further provides nucleic acid probes which are capable of specifically hybridizing (as defined below) to a PS1 gene under conditions of high stringency. Within one related aspect, such probes comprise at least a portion of the nucleotide sequence shown in Fig. 1A-Fig. 1F or Fig. 2A-Fig. 2F, or its complementary sequence, the probe being capable of specifically hybridizing to a mutant PS1 gene under conditions of high stringency. Within one particularly preferred aspect, probes are provided that are capable of specifically

hybridizing to a mutant PS1 gene encoding a protein in which amino acid residue 263 is changed from cysteine to arginine, or in which amino acid 264 is changed from proline to leucine, or in which amino acid 269 is changed from arginine to histidine, each under conditions of very high stringency. Representative probes of the present invention are generally at least 12 nucleotide bases in length, although they may be longer. Also provided are primer pairs capable of specifically amplifying all, or a portion of, any of the nucleic acid molecules disclosed herein.

[0026] Moreover, in the present invention, methods and kits are provided for diagnosing a patient having an increased likelihood of contracting Alzheimer's disease comprising the steps of: a) obtaining from a patient a biological sample containing nucleic acid, b) incubating the nucleic acid with a probe which is capable of specifically hybridizing to a mutant PS1 gene under conditions and for time sufficient to allow hybridization to occur, and c) detecting the presence of hybridized probe, and thereby determining that said patient has an increased likelihood of contracting Alzheimer's disease.

[0027] Within another embodiment, methods are provided comprising the steps of: a) obtaining from a patient a biological sample containing nucleic acid, b) amplifying selected nucleic acid sequence associated with a mutant PS1 gene, and c) detecting the presence of an amplified nucleic acid sequence, and thereby determining that the patient has an increased likelihood of contracting Alzheimer's disease.

[0028] Within yet another embodiment, methods are provided comprising the steps of: a) contacting a biological sample obtained from a patient with an antibody that specifically binds to a mutant PS1 protein under conditions and for a time sufficient to allow binding of the antibody to the protein and b) detecting the presence of the bound antibody.

[0029] The invention also extends to products useful for carrying out a method of detection, such as DNA probes (labeled or unlabeled), kits and the like. And,

the invention also provides a method of detecting a DNA segment within the Alzheimer's disease region of chromosome 14.

This invention further provides a diagnostic kit for the detection of the expression of PS1, or its immunological equivalents, which contains all the necessary reagents to carry out the previously described methods of detection.

[0030] In addition, the invention provides an assay and method of detection of the expression product of a gene from the Alzheimer's disease region of chromosome 14, which can be used prenatally to screen a fetus, or presymptomatically to screen a subject who is genetically predisposed to Alzheimer's disease based on his family history. Accordingly, this invention provides a diagnostic kit for the detection of the expression of PS1, or its immunological equivalents.

[0031] Within another embodiment of the present invention, peptide vaccines are provided which comprises a portion of a mutant PS1 gene product containing a mutation, in combination with a pharmaceutically acceptable carrier or diluent.

[0032] Within yet another aspect of the invention, transgenic animals are provided whose germ cells and somatic cells contain a PS1 gene which is operably linked to a promoter effective for the expression of the gene, the gene being introduced into the animal, or an ancestor of the animal, at an embryonic stage.

[0033] In addition, other embodiments provide expression of the PS1 gene from a vector as described above. While in yet another embodiment, the PS1 gene encodes a mutant gene product.

[0034] These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. In addition, various references are set forth herein which describe in more detail certain procedures or compositions (e.g., plasmids, etc.), and are therefore incorporated by reference their entirety.

BRIEF DESCRIPTION OF THE FIGURES

[0035] Fig. 1A-Fig. 1F depicts the nucleotide sequence of the normal S182 gene, PS1 locus (SEQ ID NO:1). Within the coding region, beneath each line of nucleotide sequence are the corresponding putative amino acid residues (SEQ ID NOs:2 and 7-26).

[0036] Fig. 2A-Fig. 2F depicts identified mutations (shown by arrows) at nucleotide sequence positions 1035, 1039 and 1054 of the S182 gene, PS1 locus (SEQ ID NO:3). Within the coding region, beneath each line of nucleotide sequence are the corresponding putative amino acid residues (SEQ ID NOs:4 and 7-26).

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0037] In the description that follows, a number of terms used in recombinant DNA (rDNA) technology are extensively utilized. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

[0038] *Abbreviations:* AD, Alzheimer's disease; APP, amyloid precursor protein gene; APLP1 and APLP2, amyloid precursor like proteins; DNA, deoxyribonucleic acid; DS, Down syndrome; EST, expression sequence tag; FAD, familial AD; PS1, the designation given to the chromosome 14 early-onset FAD gene (S182); PS2, the designation given to the chromosome 1 early-onset FAD gene at locus 14q24.31; NFTs, neurofibrillary tangles; PCR, polymerase chain reaction; RT-PCR, PCR process in which RNA is first transcribed into DNA at the first step using reverse transcriptase (RT); RNA, ribonucleic acid; SSCP, single strand conformation polymorphism analysis; STRP, short tandem repeat

polymorphism; Θ, recombination fraction; YAC, yeast artificial chromosome; Z_{max}, maximum LOD score.

[0039] A "DNA segment," refers to a molecule comprising a linear stretch of nucleotides wherein the nucleotides are present in a sequence that encodes, through the genetic code, a molecule comprising a linear sequence of amino acid residues that is referred to as a protein, a protein fragment or a polypeptide.

[0040] A "gene" is a DNA sequence related to a single polypeptide chain or protein, and as used herein includes the 5′ and 3′ ends. The polypeptide can be encoded by a full-length sequence or any portion of the coding sequence, so long as the functional activity of the protein is retained.

[0041] A "complementary DNA" or "cDNA" gene includes recombinant genes synthesized by reverse transcription of messenger RNA ("mRNA") lacking intervening sequences (introns).

[0042] A "structural gene" is a DNA sequence that is transcribed into mRNA that is then translated into a sequence of amino acids characteristic of a specific polypeptide. Typically the first nucleotide of the first translated codon is numbered +1, and the nucleotides are numbered consecutively with positive integers through the translated region of the structural gene and into the 3′ untranslated region. The numbering of the nucleotides in the promoter and regulatory region 5′ to the translated region proceeds consecutively with negative integers with the 5′ nucleotide next to the first translated nucleotide being numbered -1.

[0043] A "restriction endonuclease" (also "restriction enzyme") is an enzyme that has the capacity to recognize a specific base sequence (usually 4, 5, or 6 base pairs in length) in a double-stranded DNA molecule, and to cleave both strands of the DNA molecule at every place where this sequence appears. For example, *Eco*RI recognizes the base sequence GAATTC/CTTAAG.

[0044] A "restriction fragment" comprises the DNA molecules produced by digestion with a restriction endonuclease are referred to as restriction fragments.

Any given genome will be digested by a particular restriction endonuclease into a discrete set of restriction fragments.

[0045] "Agarose gel electrophoresis" is an analytical method for fractionating double-stranded DNA molecules on the basis of size is required. The most commonly used technique (though not the only one) for achieving such a fractionation is agarose gel electrophoresis. The principle of this method is that DNA molecules migrate through the gel as though it were a sieve that retards the movement of the largest molecules to the greatest extent and the movement of the smallest molecules to the least extent. Note that the smaller the DNA fragment, the greater the mobility under electrophoresis in the agarose gel.

[0046] The DNA fragments fractionated by agarose gel electrophoresis can be visualized directly by a staining procedure if the number of fragments included in the pattern is small. The DNA fragments of genomes can be visualized successfully. However, most genomes, including the human genome, contain far too many DNA sequences to produce a simple pattern of restriction fragments. For example, the human genome is digested into approximately 1,000,000 different DNA fragments by *Eco*RI. In order to visualize a small subset of these fragments, a methodology referred to as the Southern hybridization procedure can be applied.

[0047] "Southern blotting" or "Southern transfer" is a technique for physically transferring DNA fractionated by agarose gel electrophoresis onto a nitrocellulose filter paper or another appropriate surface or method, while retaining the relative positions of DNA fragments resulting from the fractionation procedure. The methodology used to accomplish the transfer from agarose gel to nitrocellulose involves drawing the DNA from the gel into the nitrocellulose paper by capillary action.

[0048] "Nucleic acid hybridization" depends on the principle that two single-stranded nucleic acid molecules that have complementary base sequences will reform the thermodynamically favored double-stranded structure if they are mixed in solution under the proper conditions. The double-stranded structure will be

formed between two complementary single-stranded nucleic acids even if one is immobilized on a nitrocellulose filter. In the Southern hybridization procedure, the latter situation occurs. As noted previously, the DNA of the individual to be tested is digested with a restriction endonuclease, fractionated by agarose gel electrophoresis, converted to the single-stranded form, and transferred to nitrocellulose paper, making it available for reannealing to the hybridization probe.

[0049] A "hybridization probe" (or simply a "probe") is used to visualize a particular DNA sequence in the Southern hybridization procedure, a labeled DNA molecule or hybridization probe is reacted to the fractionated DNA bound to the nitrocellulose filter. The areas on the filter that carry DNA sequences complementary to the labeled DNA probe become labeled themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling are visualized. The hybridization probe is generally produced by molecular cloning of a specific DNA sequence from the human genome.

[0050] "Oligonucleotide" or "oligomer" refers to a molecule comprised of two or more deoxyribonucleotides or ribonucleotides, preferably more than three. Its exact size will depend on many factors, which in turn depend on the ultimate function or use of the oligonucleotide. An oligonucleotide may be derived synthetically or by cloning.

[0051] "Sequence amplification" (or simply "amplification") is a method for generating large amounts of a target sequence. In general, one or more amplification primers are annealed to a nucleic acid sequence. Using appropriate enzymes, sequences found adjacent to, or in between the primers is amplified.

[0052] An "amplification primer" is an oligonucleotide capable of annealing adjacent to a target sequence and serving as an initiation point for DNA synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is initiated.

[0053] A "vector" (also a "cloning vector" or "cloning vehicle") refers to an assembly which is capable of directing the expression of the PS1 gene, as well as any additional sequence(s) or gene(s) of interest. The vector must include

transcriptional promoter elements which are operably linked to the genes of interest. The vector may be composed of a plasmid, phage DNA, or other DNA sequence, an RNA sequence, or a combination of the two (*e.g.*, a DNA-RNA chimer), which is used to "carry" inserted foreign DNA for the purpose of producing more material or protein product. The vector may replicate autonomously in a host cell, and may be characterized by one or a small number of endonuclease recognition sites at which point the DNA sequence may be cut in a determinable fashion without loss of an essential biological function of the vehicle, and into which PS1 DNA may be spliced in order to bring about its replication and cloning.

[0054] "Expression" is the process by which a structural gene produces a polypeptide. It involves transcription of the gene into mRNA, and the translation of such mRNA into polypeptide(s).

[0055] An "expression vector" is a cloning vector or vehicle designed so that a cloned gene or coding sequence inserted at a particular site will be transcribed and translated into protein. The cloned gene is placed under the control of (*i.e.*, "operably linked to") certain control sequences, such as promoter sequence(s), a polyadenylation sequence, one or more restriction sites, as well as one or more selectable markers, such as neomycin phosphotransferase, or proteins providing tetracycline or ampicillin resistance.

[0056] Expression control sequences will vary depending on whether the vector is designed to express the operably linked gene in a prokaryotic or eukaryotic host and may additionally contain an origin of replication, additional nucleic acid restriction sites, transcriptional elements, such as enhancer elements, termination sequences, tissue-specificity elements, and/or translational initiation and termination sites, sequences conferring inducibility of transcription, and other selectable markers.

[0057] The present invention pertains both to expression of a PS1 gene, and to the expression product of the gene, as well as to functional derivatives thereof.

[0058] A "functional derivative" of the PS1 sequence is a molecule that possesses a biological activity that is substantially similar to a biological activity of a the non-recombinant PS1 protein, or nucleic acid encoding it. The protein may or may not contain post-translational modifications such as a covalently linked carbohydrate, depending on the necessity of such modifications for the performance of a specific function. The term "functional derivative" is intended to include the "fragments," "segments," "variants," "analogs," or "chemical derivatives" of a molecule.

[0059] As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties may improve the molecule's solubility, absorption, biological half life, and the like. The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, and the like. Moieties capable of mediating such effects are disclosed in *Remington's Pharmaceutical Sciences* (1980). Procedures for coupling such moieties to a molecule are well known in the art.

[0060] A "fragment" of a protein or nucleic acid molecule is meant to refer to any portion of a native PS1 amino acid or nucleotide genetic sequence.

[0061] A "variant" of a PS1 protein or nucleic acid is meant to refer to a molecule substantially similar in structure and biological activity to either a native PS1 protein, or to a fragment thereof. Thus, provided that two molecules possess a common activity and may substitute for each other, they are considered variants as that term is used herein even if the composition or secondary, tertiary, or quaternary structure of one of the molecules is not identical to that found in the other, or if the amino acid or nucleotide sequence is not identical.

[0062] An "analog" of a PS1 protein or genetic sequence is meant to refer to a protein or genetic sequence which is substantially similar in function to the PS1 sequence described herein. For example, analogs of a PS1 protein described herein include isozymes and analogs of the PS1 protein or genetic sequences described herein, including alleles of the PS1 protein molecule.

[0063] An "allele" is an alternative form of a gene. In most organisms there are two alleles of any one gene (one from each parent) which occupy the same relative position on homologous chromosomes. Homozygous organisms have two identical alleles controlling a particular feature (these may be either dominant or recessive). Heterozygous organisms have two different alleles controlling a particular feature. The aspect of the feature displayed by the organism will be that determined by the dominant allele.

[0064] A "substantially pure" PS1 protein is a preparation generally lacking other cellular components, especially other non-Alzheimer's disease-linked peptides or nucleic acids.

[0065] A "genetic marker" is any segment of a chromosome that is distinguishably unique in the genome, and polymorphic in the population so as to provide information about the inheritance of linked DNA sequences, genes and/or other markers.

[0066] "Autosomal dominant" means that a trait is encoded on one of the non-sex chromosomes (autosomes) and is dominant for the phenotype it dictates for an individual having a heterozygous state.

[0067] "LOD score" is a standard measure in genetics of the likelihood of a trait being localized in the interval being scored. It is the logarithm of a calculated probability.

[0068] "Early onset Alzheimer's disease" is commonly understood to mean onset (the patient displays recognized clinical symptoms indicating AD) before age 65. By comparison "familial Alzheimer's Disease" (FAD) is a subcategory of early-onset AD, in which the genetic relationship is established because at least two of the patient's first degree relatives have presented confirmed clinical symptoms of AD at approximately the same age of early onset as the patient.

Preferred Embodiments of the Invention

[0069] The present invention relates to novel methods and compositions for the detection and treatment of Alzheimer's disease. These methods and compositions are based upon the discovery that certain mutations of the S182 gene for AD on chromosome 14 increases the probability of Alzheimer's Disease.

I. Isolated Nucleic Acid Molecules Coding for PS1 Polypeptides

[0070] At its broadest, the invention comprises a nucleic acid sequence encompassing at least one mutation of the PS1 (S182) gene for AD on human chromosome 14. In particular, the isolated DNA segment encodes expression products useful in determining the normal role of the PS1 (S182) gene, and for developing experimental and animal models addressing the mechanisms by which alterations of PS1 influence or cause AD.

A. Isolation of Nucleic Acid

[0071] Although one embodiment of the mutant PS1 gene is disclosed in Fig. 2A-Fig. 2F, it should be understood that the present invention is not so limited. In particular, within the context of the present invention reference to the PS1 gene should be understood to include derivatives, analogs, or allelic variants of the gene disclosed in Fig. 1A-Fig. 1F that are substantially similar. As used herein, a nucleic acid molecule is deemed to be "substantially similar" if (a) the nucleotide sequence is derived from the coding region of the described gene and includes portions of the sequence or allelic variations of the sequences discussed above; (b) the nucleotide sequence is capable of hybridization to nucleotide sequences of the present invention under high or very high stringency (see Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989)); or (c) the DNA sequences are

degenerate as a result of the genetic code to the DNA sequences defined in (a) or (b).

[0072] Further, the PS1 gene includes both complementary and non-complementary sequences, provided the sequences otherwise meet the criteria set forth herein. Within the context of the present invention, high stringency means standard hybridization conditions (e.g., 5x SSPE, 0.5% SDS at 65°C, or the equivalent), such that an appropriate nucleotide sequence is able to electively hybridize to nucleotide sequences from the AD-related gene, and to mutant nucleotide sequences. Very high stringency means the nucleotide sequence is able to selectively hybridize to a single allele of the AD-related gene.

[0073] The PS1 gene is isolated from genomic DNA or cDNA. The DNA segment may be isolated from a biological sample, preferably a biological sample containing nucleated cells. Most preferably the nucleated cells are obtained from a human. Genomic DNA libraries constructed in vectors, such as YACs (yeast artificial chromosomes), bacteriophage vectors, such as λEMBL3, λgt10, cosmids or plasmids, are suitable for screening, as are cDNA libraries constructed in bacteriophage vectors, plasmids, or the like. Such libraries may be constructed using methods and techniques known in the art (see Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989)) or purchased from commercial sources (*e.g.*, Clontech).

[0074] Alternately, the PS1 gene may be isolated by PCR methods from genomic DNA, cDNA or libraries, or by probe hybridization of genomic DNA or cDNA libraries. Primers for PCR and probes for hybridization screening may be designed based on the DNA sequence of PS1 presented herein. The DNA sequence of PS1 and the corresponding predicted amino acid sequence of PS1 is presented in Fig. 1A-Fig. 1F. Primers for PCR should be derived from sequences in the 5′ and 3′ untranslated region in order to isolate a full-length cDNA. The primers should not have self-complementary sequences nor have complementary sequences at their 3′ end (to prevent primer-dimer formation). Preferably, the GC content of the primers is about 50% and contain restriction sites. The primers are annealed

to cDNA and sufficient cycles of PCR are performed to yield a product readily visualized by gel electrophoresis and staining. Mutations can be visualized by single strand conformation polymorphism (SSCP) analysis. The amplified fragment is purified and inserted into a vector, such as λgt10 or pBS(M13+), and propagated.

[0075] Suitable biological samples having nucleated cells that may be used in this invention include, but are not limited to, peripheral blood, buccal swabs, or brain tissue. The method of obtaining the biological sample will vary depending upon the nature of the sample. Such cells may either be normal or neoplastic.

B. Synthesis of Nucleic Acid

[0076] The DNA segment of the present invention may also be chemically synthesized according to the methods and techniques known to those skilled in the art. For example, a DNA fragment with the nucleotide sequence which codes for the modified expression product of the PS1 gene may be designed and, if necessary, divided into appropriate smaller fragments. Then an oligomer which corresponds to the DNA fragment, or to each of the divided fragments, may be synthesized. Such synthetic oligonucleotides may be prepared, for example, by the triester method of Matteucci *et al.*, *J. Am. Chem. Soc.* 103:3185-3191 (1981) or by using an automated DNA synthesizer.

[0077] An oligonucleotide hybridization probe suitable for screening genomic or cDNA libraries may be designed based on the sequence provided herein. Preferably, the oligonucleotide is 20-30 bases long. Such an oligonucleotide may be synthesized by automated synthesis. The oligonucleotide may be conveniently labeled at the 5′ end with a reporter molecule, such as a radionuclide, (*e.g.*, ^{32}P) or biotin. The library is plated as colonies or phage, depending upon the vector, and the recombinant DNA is transferred to nylon or nitrocellulose membranes. Following denaturation, neutralization, and fixation of the DNA to the membrane, the membranes are hybridized with the labeled probe. The membranes are washed

and the reporter molecule detected. The hybridizing colonies or phage are isolated and propagated. Candidate clones or PCR amplified fragments may be verified as containing PS1 DNA by any of various means. For example, mutations can be visualized by single strand conformation polymorphism (SSCP) analysis. Alternately, candidate clones may be hybridized with a second, nonoverlapping probe or subjected to DNA sequence analysis. In these ways, clones containing PS1 gene, which are suitable for use in the present invention, are isolated.

II. Substantially Pure PS1 Polypeptides

[0078] In another embodiment, the present invention relates to a substantially pure polypeptide having an amino acid sequence corresponding to PS1 or a mutant thereof. In a preferred embodiment, the polypeptide has specific mutation(s) in which amino acid residue 263 is changed from cysteine to arginine, or in which amino acid 264 is changed from proline to leucine, or in which amino acid 269 is changed from arginine to histidine. The present invention also relates to fragments of the PS1 polypeptide and mutants thereof that exhibit similar activity to that exhibited by PS1 as measured in a particular biological assay.

[0079] A variety of methodologies known in the art can be utilized to obtain the peptide of the present invention. The structure of the proteins encoded by the nucleic acid molecules described herein may be predicted from the primary translation products using the hydrophobicity plot function of, for example, P/C Gene or Intelligenetics Suite (Intelligenetics, Mountain View, Calif.), or according to the methods described by Kyte and Doolittle (*J. Mol. Biol 157*:105-132 (1982)).

[0080] There are a variety of sources encoding a peptide. The peptide can be isolated as described herein from any source having the PS1 peptide. Preferably, the peptide can be isolated from a mammalian source, most preferably from a human source. In the alternative, the sequence encoding the peptide can be

synthesized by methods known in the art or expressed by methods disclosed herein.

[0081] As used herein, a cell is said to be "altered to express a desired peptide" when the cell, through genetic manipulation, is made to produce a protein which it normally does not produce or which the cell normally produces at low levels. One skilled in the art can readily adapt procedures for introducing and expressing either genomic, cDNA, or synthetic sequences into either eukaryotic or prokaryotic cells in order to generate a cell which produces the peptide, in particular the PS1 peptide.

[0082] PS1 proteins of the present invention may be in the form of acidic or basic salts, or in neutral form. In addition, individual amino acid residues may be modified by oxidation or reduction. Furthermore, various substitutions, deletions, or additions may be made to the amino acid or nucleic acid sequences, the net effect of which is to retain or further enhance or decrease the biological activity of the mutant or wild-type protein. Moreover, due to degeneracy in the genetic code, there may be considerable variation in nucleotide sequences encoding the same amino acid sequence.

[0083] Guidance as to how to make phenotypically silent amino acid substitutions is provided in J.U. Bowie *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247:*1306-1310 (1990), wherein the authors indicate that there are two main approaches for studying the tolerance of an amino acid sequence to change. The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection. The second approach uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene and selections or screens to identify sequences that maintain functionality. As the authors state, these studies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at a certain position of the protein. For example, most buried amino acid residues require nonpolar side chains, whereas few features of surface side chains are

generally conserved. Other such phenotypically silent substitutions are described in Bowie, J.U. *et al., supra,* and the references cited therein.

[0084] Other derivatives of the PS1 proteins disclosed herein include conjugates of the proteins along with other proteins or polypeptides. This may be accomplished, for example, by the synthesis of N-terminal or C-terminal fusion proteins which may be added to facilitate purification or identification of Alzheimer Disease Proteins (*see* U.S. Patent No. 4,851,341; *see also*, Hopp *et al.*, *Biotechnology 6*:1204 (1988). Alternatively, fusion proteins such as PS1-β-galactosidase or PS1-luciferase may be constructed in order to assist in the identification, expression, and analysis of the PS1 proteins.

[0085] PS1 proteins of the present invention may be constructed using a wide variety of techniques, including those set forth in the Examples. Further, mutations may be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes a derivative having the desired amino acid insertion, substitution, or deletion.

[0086] Alternatively, oligonucleotide-directed site-specific (or segment specific) mutagenesis procedures may be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the alterations set forth above are disclosed by Walder *et al.* (*Gene 42*:133 (1986)); Bauer *et al.* (*Gene 37*:73 (1985)); Craik (*BioTechniques*, January 1985, pp. 12-19); Smith *et al.* (*Genetic Engineering: Principles and Methods*, Plenum Press, New York, NY (1981); and Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, *supra*. Deletion or truncation derivatives of PS1 proteins (*e.g.*, a soluble extracellular portion) may also be constructed by utilizing convenient restriction endonuclease sites adjacent to the desired deletion. Subsequent to restriction, overhangs may be filled in, and the DNA relegated. Exemplary methods of making the alterations set forth above

are disclosed by Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, *supra*.

[0087] Mutations which are made in the nucleic acid molecules of the present invention preferably preserve the reading frame of the coding sequences. Furthermore, the mutations will preferably not create complementary regions that could hybridize to produce secondary mRNA structures, such as loops or hairpins, that would adversely affect translation of the mRNA. Although a mutation site may be predetermined, it is not necessary that the nature of the mutation *per se* be predetermined. For example, in order to select for optimum characteristics of mutants at a given site, random mutagenesis may be conducted at the target codon and the expressed mutants screened for indicative biological activity. Alternatively, mutations may be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes a derivative having the desired amino acid insertion, substitution, or deletion.

[0088] PS1 proteins may also be constructed utilizing techniques of PCR mutagenesis, chemical mutagenesis (Drinkwater and Klinedinst, *Proc. Natl. Acad. Sci. USA 83*:3402-3406 (1986)), by forced nucleotide misincorporation (*e.g.*, Liao and Wise, *Gene 88*:107-111 (1990)), or by use of randomly mutagenized oligonucleotides (Horwitz *et al.*, *Genome 3*:112-117 (1989)). Particularly preferred methods for constructing Alzheimer's disease-related proteins are set forth in more detail in the Examples.

[0089] In another aspect, the present invention provides a peptide or polypeptide comprising an epitope-bearing portion of the PS2 polypeptide or a mutant thereof. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. These immunogenic epitopes are believed to be confined to a few loci on the molecule. On the other hand, a region of a protein molecule to which

an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. *See, e.g.*, Geysen *et al., Proc. Natl. Acad. Sci. USA 81*:3998- 4002 (1983).

[0090] As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. *See, e.g.*, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R.A., *Science 219*:660-666 (1983). Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (*i.e.*, immunogenic epitopes) nor to the amino or carboxyl terminals. Peptides that are extremely hydrophobic and those of six or fewer residues generally are ineffective at inducing antibodies that bind to the mimicked protein; longer, peptides, especially those containing proline residues, usually are effective. Sutcliffe *et al., supra*, at 661. For instance, 18 of 20 peptides designed according to these guidelines, containing 8-39 residues covering 75% of the sequence of the influenza virus hemagglutinin HA1 polypeptide chain, induced antibodies that reacted with the HA1 protein or intact virus; and 12/12 peptides from the MuLV polymerase and 18/18 from the rabies glycoprotein induced antibodies that precipitated the respective proteins.

[0091] Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. Thus, a high proportion of hybridomas obtained by fusion of spleen cells from donors immunized with an antigen epitope-bearing peptide generally secrete antibody reactive with the native protein. Sutcliffe *et al., supra*, at 663. The antibodies raised by antigenic epitope-bearing peptides or polypeptides are useful to detect the mimicked

protein, and antibodies to different peptides may be used for tracking the fate of various regions of a protein precursor which undergoes post-translational processing. The peptides and anti-peptide antibodies may be used in a variety of qualitative or quantitative assays for the mimicked protein, for instance in competition assays since it has been shown that even short peptides (*e.g.*, about 9 amino acids) can bind and displace the larger peptides in immunoprecipitation assays. *See, e.g.*, Wilson *et al., Cell 37*:767-778 (1984). The anti- peptide antibodies of the invention also are useful for purification of the mimicked protein, for instance, by adsorption chromatography using methods well known in the art.

[0092] Antigenic epitope-bearing peptides and polypeptides of the invention designed according to the above guidelines preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of a polypeptide of the invention, containing about 30 to about 50 amino acids, or any length up to and including the entire amino acid sequence of a polypeptide of the invention, also are considered epitope-bearing peptides or polypeptides of the invention and also are useful for inducing antibodies that react with the mimicked protein. Preferably, the amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (*i.e.*, the sequence includes relatively hydrophilic residues and highly hydrophobic sequences are preferably avoided); and sequences containing proline residues are particularly preferred.

[0093] The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means for making peptides or polypeptides including recombinant means using nucleic acid molecules of the invention. For instance, a short epitope-bearing amino acid sequence may be fused to a larger polypeptide which acts as a carrier during recombinant production and purification, as well as during immunization to produce anti-peptide antibodies. Epitope-bearing peptides also may be synthesized using known methods of

chemical synthesis. For instance, Houghten has described a simple method for synthesis of large numbers of peptides, such as 10-20 mg of 248 different 13 residue peptides representing single amino acid variants of a segment of the HA1 polypeptide which were prepared and characterized (by ELISA-type binding studies) in less than four weeks. Houghten, R. A. (1985) General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. USA 82*:5131-5135. This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986). In this procedure the individual resins for the solid-phase synthesis of various peptides are contained in separate solvent-permeable packets, enabling the optimal use of the many identical repetitive steps involved in solid-phase methods. A completely manual procedure allows 500-1000 or more syntheses to be conducted simultaneously. Houghten *et al.*, *supra*, at 5134.

[0094] Immunogenic epitope-bearing peptides of the invention, *i.e.*, those parts of a protein that elicit an antibody response when the whole protein is the immunogen, are identified according to methods known in the art. For instance, Geysen *et al.*, *supra*, discloses a procedure for rapid concurrent synthesis on solid supports of hundreds of peptides of sufficient purity to react in an enzyme-linked immunosorbent assay. Interaction of synthesized peptides with antibodies is then easily detected without removing them from the support. In this manner a peptide bearing an immunogenic epitope of a desired protein may be identified routinely by one of ordinary skill in the art. For instance, the immunologically important epitope in the coat protein of foot-and-mouth disease virus was located by Geysen *et al.* with a resolution of seven amino acids by synthesis of an overlapping set of all 208 possible hexapeptides covering the entire 213 amino acid sequence of the protein. Then, a complete replacement set of peptides in which all 20 amino acids were substituted in turn at every position within the epitope were synthesized, and the particular amino acids conferring specificity for the reaction with antibody were determined. Thus, peptide analogs of the epitope-bearing peptides of the

invention can be made routinely by this method. U.S. Patent No. 4,708,781 to Geysen (1987) further describes this method of identifying a peptide bearing an immunogenic epitope of a desired protein.

[0095] Further still, U.S. Patent No. 5,194,392 to Geysen (1990) describes a general method of detecting or determining the sequence of monomers (amino acids or other compounds) which is a topological equivalent of the epitope (*i.e.*, a "mimotope") which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More generally, U.S. Patent No. 4,433,092 to Geysen (1989) describes a method of detecting or determining a sequence of monomers which is a topographical equivalent of a ligand which is complementary to the ligand binding site of a particular receptor of interest. Similarly, U.S. Patent No. 5,480,971 to Houghten, R. A. *et al.* (1996) on Peralkylated Oligopeptide Mixtures discloses linear C_1-C_7-alkyl peralkylated oligopeptides and sets and libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated oligopeptide that preferentially binds to an acceptor molecule of interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also can be made routinely by these methods.

III. Recombinant Expression of PS1

[0096] The present invention also provides for the manipulation and expression of the above-described genes by culturing host cells containing a vector capable of expressing the above-described genes. Such vectors or vector constructs include either synthetic or cDNA-derived nucleic acid molecules encoding PS1 proteins, which are "operably linked" to suitable transcriptional or translational regulatory elements.

[0097] The precise nature of the regulatory regions needed for gene sequence expression may vary from organism to organism, but shall, in general, include a promoter region which, in prokaryotes, contains both the promoter (which directs

the initiation of RNA transcription) and the DNA sequences, which when transcribed into RNA will signal synthesis initiation. Such regions will normally include those 5'-non-coding sequences involved with initiation of transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like.

[0098] Proper expression in a prokaryotic cell also requires the presence of a ribosome binding site upstream of the gene sequence-encoding sequence. Such ribosome binding sites are disclosed, for example, by Gold *et al.* (*Ann. Rev. Microbiol. 35*:365-404 (1981)).

[0099] Suitable regulatory elements may be derived from a variety of sources, including bacterial, fungal, viral, mammalian, insect, or plant genes. Selection of appropriate regulatory elements is dependent on the host cell chosen, and may be readily accomplished by one of ordinary skill in the art. Examples of regulatory elements include: a transcriptional promoter and enhancer or RNA polymerase binding sequence, a transcriptional terminator, and a ribosomal binding sequence, including a translation initiation signal.

[0100] A PS1 protein encoded by any nucleic acid molecules described above may be readily expressed by a wide variety of prokaryotic and eukaryotic host cells, including bacterial, mammalian, insect, yeast or other fungi, viral, insect, or plant cells. Methods for transforming or transfecting such cells to express foreign DNA are well known in the art.

[0101] The genetic coding sequence, *e.g.*, PS1, and an operably linked promoter may be introduced into a recipient prokaryotic or eukaryotic cell either as a non-replicating DNA (or RNA) molecule, which may either be a linear molecule or, more preferably, a closed covalent circular molecule. Since such molecules are incapable of autonomous replication, the expression of the gene may occur through the transient expression of the introduced sequence. Alternatively, permanent expression may occur through the integration of the introduced DNA sequence into the host chromosome.

[0102] In one embodiment, a vector is employed which is capable of integrating the desired gene sequences into the host cell chromosome. Cells which have stably integrated the introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker may provide for prototrophy to an auxotrophic host, biocide resistance, *e.g.*, antibiotics, or heavy metals, such as copper, or the like. The selectable marker gene sequence can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements may also be needed for optimal synthesis of single chain binding protein mRNA. These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals.

[0103] If desired, the non-coding region 3′ to the sequence encoding a PS1 gene may be included for its transcriptional termination regulatory sequences, such as termination and polyadenylation. Thus, by retaining the 3′-region naturally contiguous to the DNA sequence encoding the gene, the transcriptional termination signals may be provided. Where the transcriptional termination signals are not satisfactorily functional in the expression host cell, then a 3′ region functional in the host cell may be substituted.

A. Vectors

[0104] Bacterial expression vectors preferably comprise a promoter which functions in the host cell, one or more selectable phenotypic markers, and a bacterial origin of replication. Representative promoters include the β-lactamase (penicillinase) and lactose promoter system (see Chang *et al.*, *Nature 275*:615 (1978)), the T7 RNA polymerase promoter (Studier *et al.*, *Meth. Enzymol. 185*:60-89 (1990)), the lambda promoter (Elvin *et al.*, *Gene 87*:123-126 (1990)), the *trp* promoter (Nichols & Yanofsky, *Meth. in Enzymology 101*:155 (1983)) and the *tac* promoter (Russell *et al.*, *Gene 20*:231 (1982)). Representative selectable

markers include various antibiotic resistance markers such as the kanamycin or ampicillin resistance genes.

[0105] Many plasmids suitable for transforming host cells are well known in the art, including among others, pBR322 (see Bolivar *et al.*, *Gene 2*:95 (1977)), the pUC plasmids pUC18, pUC19, pUC118, pUC119 (see Messing, *Meth. in Enzymology 101*:20-77, 1983) and Vieira & Messing, *Gene 19*:259-268 (1982)), and pNH8A, pNH16a, pNH18a, and Bluescript M13 (Stratagene, La Jolla, Calif).

[0106] Suitable expression vectors for yeast and fungi include, among others, YCp50 (ATCC No. 37419) for yeast, and vectors pV3 (Turnbull, *Bio/Technology 7*:169 (1989)), YRp7 (Struhl *et al.*, *Proc. Natl. Acad. Sci. USA 76*:1035-1039 (1978)), YEp13 (Broach *et al.*, *Gene 8*:121-133 (1979)), pJDB249 and pJDB219 (Beggs, *Nature 275*:104-108 (1978)), and derivatives thereof.

[0107] Preferred promoters for use in yeast include promoters from yeast glycolytic genes (Hitzeman *et al.*, J. *Biol. Chem. 255*:12073-12080 (1980); Alber & Kawasaki, *J. Mol. Appl. Genet. 1*:419-434 (1982)) or alcohol dehydrogenase genes (Ammerer, *Meth. Enzymol.* 101:192-201 (1983). Examples of useful promoters for fungi vectors include those derived from *Aspergillus nidulans* glycolytic genes, such as the *adh3* promoter (McKnight *et al.*, *EMBO J. 4*:2093-2099 (1985)). The expression units may also include a transcriptional terminator. An example of a suitable terminator is the *adh3* terminator (McKnight *et al.*, *EMBO J. 4*:2093-2099 (1985)).

[0108] As with bacterial vectors, the yeast vectors will generally include a selectable marker, which may be one of any number of genes that exhibit a dominant phenotype for which a phenotypic assay exists to enable transformants to be selected. Preferred selectable markers are those that complement host cell auxotrophy, provide antibiotic resistance or enable a cell to utilize specific carbon sources, and include *leu2* (Broach *et al.*, *Gene 8*:121-133 (1979)), *ura3* (Botstein *et al.*, *Gene 8*:17 (1979)), or *his3* (Struhl *et al.*, *Proc. Natl. Acad. Sci. USA 76*:1035-1039 (1978)). Another suitable selectable marker is the gene conferring chloramphenicol resistance on yeast cells.

[0109] Techniques for transforming fungi are well known in the literature, and have been described, for instance, by Beggs (*Nature 275*:104-108 (1978)), Hinnen *et al.* (*Proc. Natl. Acad. Sci. USA 75*:1929-1933 (1978)), Yelton *et al.* (*Proc. Natl. Acad. Sci. USA 81*:1740-1747 (1984)), and Russell (*Nature 301*:167-169, 1983)). The genotype of the host cell may contain a genetic defect that is complemented by the selectable marker present on the expression vector. Choice of a particular host and selectable marker is well within the level of ordinary skill in the art.

[0110] Viral vectors include those which comprise a promoter that directs the expression of an isolated nucleic acid molecule that encodes an Alzheimer disease protein. A wide variety of promoters may be utilized within the context of the present invention, including for example, promoters such as MoMLV LTR, RSV LTR, Friend MuLV LTR, adenoviral promoter (Ohno *et al.*, *Science 265*:781-784 (1994)), neomycin phosphotransferase promoter/enhancer, late parvovirus promoter (Koering *et al.*, *Hum. Gene Therap. 5*:463 (1994)), herpes tk promoter, SV40 promoter, metallothionein enhancer/promoter, cytomegalovirus immediate early promoter, and the cytomegalovirus immediate late promoter.

[0111] Within particularly preferred embodiments of the invention, the promoter is a tissue-specific promoter (*see e.g.*, WO 91/02805; EP 0,415,731; and WO 90/07936). Representative examples of suitable tissue specific promoters include neural specific enolase promoter, platelet derived growth factor β promoter, bone morphogenetic protein promoter, human α-l-chimaerin promoter, synapsin I promoter and synapsin II promoter.

[0112] In addition, other viral-specific promoters (*e.g.*, retroviral promoters (including those noted above, and others, such as HIV promoters), hepatitis, herpes (*e.g.*, EBV), and bacterial, fungal or parasitic (*e.g.*, malarial)-specific promoters may be utilized in order to target a specific cell or tissue which is infected with a virus, bacteria, fungus or parasite. Thus, PS1 proteins of the present invention may be expressed from a variety of viral vectors. Within various

embodiments, either the viral vector itself, or a viral particle which contains the viral vector may be utilized in the methods and compositions described below.

[0113] Mammalian expression vectors for use in carrying out the present invention will include a promoter capable of directing the transcription of a cloned gene or cDNA. Preferred promoters include viral promoters and cellular promoters. Viral promoters include the cytomegalovirus immediate early promoter (Boshart *et al.*, *Cell 41*:521-530 (1985)), cytomegalovirus immediate late promoter, SV40 promoter (Subramani *et al.*, *Mol. Cell. Bio. 1*:854-864 (1981)), MMTV LTR, RSV LTR, metallothionein-1, adenovirus E1a.

[0114] Cellular promoters include the mouse metallothionein-1 promoter (Palmiter *et al.*, U.S. Patent No. 4,579,821), a mouse V_K promoter (Bergman *et al.*, *Proc. Natl. Acad. Sci. USA 81*:7041-7045 (1983); Grant *et al.*, *Nucl. Acids Res. 15*:5496, 1987) and a mouse V_H promoter (Loh *et al.*, *Cell 33*:85-93 (1983)). The choice of promoter will depend, at least in part, upon the level of expression desired or the recipient cell line to be transfected.

[0115] Expression vectors may also contain a set of RNA splice sites located downstream from the promoter and upstream from the DNA sequence encoding the peptide or protein of interest. Preferred RNA splice sites may be obtained from adenovirus and/or immunoglobulin genes. Also contained in the expression vectors is a polyadenylation signal located downstream of the coding sequence of interest. Suitable polyadenylation signals include the early or late polyadenylation signals from SV40, the polyadenylation signal from the adenovirus 5 EIB region and the human growth hormone gene terminator (DeNoto *et al.*, *Nuc. Acids Res. 9*:3719-3730 (1981)). The expression vectors may include a noncoding viral leader sequence, such as the adenovirus-2 tripartite leader, located between the promoter and the RNA splice sites. Preferred vectors may also include enhancer sequences, such the SV40 enhancer and the mouse I enhancer (Gillies, *Cell 33*:717-728, 1983)). Suitable expression vectors can be obtained from commercial sources (*e.g.*, Stratagene, La Jolla, Calif).

[0116] Vectors of the present invention may contain or express a wide variety of additional nucleic acid molecules in place of or in addition to a PS1 protein as described above, either from one or several separate promoters. For example, the viral vector may express a lymphokine or lymphokine receptor, antisense or ribozyme sequence or toxins. Representative examples of lymphokines include IL-1 through IL-15, GM-CSF, G-CSF, M-CSF, α-, β-, or gamma-interferon, and tumor necrosis factors, as well as their respective receptors. Representative examples of antisense sequences include antisense sequences which block the expression of PS1 protein mutants. Representative examples of toxins include: ricin, abrin, diphtheria toxin, cholera toxin, gelonin, pokeweed antiviral protein, tritin, *Shigella* toxin, and *Pseudomonas* exotoxin A.

B. Host Cells

1. Prokaryotic Host Cells

[0117] Preferred prokaryotic host cells for use within the present invention include *E. coli*, *Salmonella*, *Bacillus*, *Shigella*, *Pseudomonas*, *Streptomyces*, *Streptomyces*, and *Staphylococcus*, as well as many other bacterial genera or species well known to one of ordinary skill in the art. Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well known in the art (*see, e.g.,* Maniatis *et al., supra).* Vectors used for expressing cloned DNA sequences in bacterial hosts will generally contain a selectable marker, such as a gene for antibiotic resistance, and a promoter that functions in the host cell. Appropriate promoters include the *trp* (Nichols & Yanofsky, *Meth. Enzymol. 101*:155-164 (1983)), *lac* (Casadaban *et al., J. Bacterio. 143*:971-980 (1980)), and phage λ (Queen, *J. Mol. Appl. Genet. 2*:1-10 (1983)) promoter systems.

[0118] Plasmids useful for transforming bacteria include the pUC plasmids (Messing, *Meth. Enzymol. 101*:20-78 (1983); Vieira & Messino,, *Gene 19*:259-268 (1982)), pBR322 (Bolivar *et al., Gene 2*:95-113 (1977)), pCQV2 (Queen, *J.*

Mol. Appl. Genet. 2:1-10 (1983)), and derivatives thereof. Plasmids may contain both viral and bacterial elements.

2. Culture Conditions

[0119] Host cells containing vector constructs of the present invention are then cultured to express a DNA molecule as described above. The cells are cultured according to standard methods in a culture medium containing nutrients required for growth of the chosen host cells. A variety of suitable media are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals, as well as other components, *e.g.*, growth factors or serum, that may be required by the particular host cells. The growth medium will generally select for cells containing the DNA construct(s) by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker on the DNA construct or co-transfected with the DNA construct.

[0120] Suitable growth conditions for yeast cells, for example, include culturing in a chemically defined medium, comprising a nitrogen source, which may be a non-amino acid nitrogen source or a yeast extract, inorganic salts, vitamins and essential amino acid supplements at a temperature between 4°C and 37°C, with 30°C being particularly preferred.

[0121] The pH of the medium is preferably maintained at a pH greater than 2 and less than 8, more preferably pH 5-6. Methods for maintaining a stable pH include buffering and constant pH control. Preferred agents for pH control include sodium hydroxide. Preferred buffering agents include succinic acid and Bis-Tris (Sigma Chemical Co., St. Louis, MO).

[0122] Due to the tendency of yeast host cells to hyperglycosylate heterologous proteins, it may be preferable to express the nucleic acid molecules of the present invention in yeast cells having a defect in a gene required for asparagine-linked glycosylation. Such cells are preferably grown in a medium containing an osmotic

stabilizer. A preferred osmotic stabilizer is sorbitol supplemented into the medium at a concentration between 0.1 M and 1.5 M, preferably at 0.5 M or 1.0 M.

3. Eukaryotic Host Cells

[0123] Preferred eukaryotic host cells include cultured mammalian cell lines (*e.g.*, rodent or human cell lines) and fungal cells, including species of yeast, or filamentous fungi. In general, a host cell will be selected on the basis of its ability to produce the protein of interest at a high level or its ability to carry out at least some of the processing steps necessary for the biological activity of the protein. In this way, the number of cloned DNA sequences that must be introduced into the host cell can be minimized and overall yield of biologically active protein can be maximized.

[0124] Any of a series of yeast gene sequence expression systems can be utilized which incorporate promoter and termination elements from the actively expressed gene sequences coding for glycolytic enzymes are produced in large quantities when yeast are grown in mediums rich in glucose. Known glycolytic gene sequences can also provide very efficient transcriptional control signals.

[0125] Yeast provides substantial advantages in that it can also carry out post-translational peptide modifications. A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number of plasmids which can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences on cloned mammalian gene sequence products and secretes peptides bearing leader sequences (i.e., pre-peptides).

[0126] Yeast and fungi host cells suitable for carrying out the present invention include, among others, *Saccharomyces pombe*, *Saccharomyces cerevisiae*, the genera *Pichia* or *Kluyveromyces* and various species of fungi (*e.g.*, genera *Aspergillus* or *Neurospora*).

[0127] Protocols for the transformation of yeast are well known to those ordinary skill in the art. For example, transformation may be readily accomplished either

by preparation of spheroplasts of yeast with DNA (see Hinnen *et al.*, *Proc. Natl. Acad. Sci. USA 75*:1929 (1978)) or by treatment with alkaline salts such as LiCl (see Itoh *et al.*, *J. Bacteriology 153*:163 (1983)). Transformation of fungi may also be carried out using polyethylene glycol as described by Cullen *et al.* (*Bio/Technology 5*:369 (1987)).

[0128] In the alternative, nucleic acid molecules which encode the PS1 proteins of the present invention (or the vectors which contain and/or express related mutants) may readily be introduced into cells from a vertebrate or warm-blooded animal, such as a human, macaque, dog, cow, horse, pig, sheep, rat, hamster, mouse or fish cell, or any hybrid thereof.

[0129] Mammalian cells which may be useful as hosts include, among others: PCI2, NIE-115 neuroblastoma, SK-N-BE(2)C neuroblastoma, SHSY5 adrenergic neuroblastoma, NS20Y and NG108-15 murine cholinergic cell lines, or rat F2 dorsal root ganglion line, COS (*e.g.*, deposited with the American Type Culture Collection (ATCC) No. CRL 1650 or 1651), BHK (e.g., ATCC No. CRL 6281; BHK 570 cell line (ATCC) under accession number CRL 10314), CHO (ATCC No. CCL 61), HeLa (e.g., ATCC No. CCL 2), 293 (ATCC No. 1573); Graham *et al.*, *J. Gen. Virol. 36*:59-72 (1977)) and NS-1 cells. Other mammalian cell lines may be used within the present invention, including Rat Hep I (ATCC No. CRL 1600), Rat Hep II (ATCC No. CRL 1548), TCMK (ATCC No. CCL 139), Human lung (ATCC No. CCL 75.1), human hepatoma (ATCC No. HTB-52), Hep G2 (ATCC No. HB 8065), mouse liver (ATCC No. CCL 29.1), NCTC 1469 (ATCC No. CCL 9.1), SP2/0-Agl4 (ATCC No. 1581), HIT-TI5 (ATCC No. CRL 1777), and RINm 5AHT$_2$B (Orskov & Nielson, *FEBS 229*(1):175-178 (1988)).

[0130] Cultured mammalian cells are generally cultured in commercially available serum-containing or serum-free media. Selection of a medium and growth conditions appropriate for the particular cell line used is well within the level of ordinary skill in the art.

[0131] Protocols for the transfection of mammalian cells are well known to those of ordinary skill in the art. Vector constructs comprising cloned DNA sequences can be introduced into cultured mammalian cells by, for example, calcium phosphate-mediated transfection (Wigler *et al.*, *Cell 14*:725 (1978); Corsaro & Pearson, *Somatic Cell Genetics 7*:603 (1981); Graham & Van der Eb, *Virology 52*:456 (1973), electroporation (Neumann *et al.*, *EMBO J. 1*:841-845 (1982), or DEAE-dextran mediated transfection (*Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley and Sons, Inc., New York, NY (1987)). To identify cells that have stably integrated the cloned DNA, a selectable marker is generally introduced into the cells along with the gene or cDNA of interest. Preferred selectable markers for use in cultured mammalian cells include genes that confer resistance to drugs, such as neomycin, hygromycin, and methotrexate. The selectable marker may be an amplifiable selectable marker. Preferred amplifiable selectable markers are the DHFR gene and the neomycin resistance gene.

[0132] Mammalian cells containing a suitable vector are allowed to grow for a period of time, typically 1-2 days, to begin expressing the DNA sequence(s) of interest. Drug selection is then applied to select for growth of cells that are expressing the selectable marker in a stable fashion. For cells that have been transfected with an amplifiable, selectable marker the drug concentration may be increased in a stepwise manner to select for increased copy number of the cloned sequences, thereby increasing expression levels. Cells expressing the introduced sequences are selected and screened for production of the protein of interest in the desired form or at the desired level. Cells that satisfy these criteria can then be cloned and scaled up for production.

[0133] In addition, plant cells are also available as hosts, and control sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. *See, e.g.,* Czako & Marton, *Plant Physiol. 104*:1067-1071 (1994); and Paszkowski *et al.*, *Biotech. 24*:387-392 (1992). For example, the use of *Agrobacterium rhizogenes* as vectors for expressing genes in

plant cells has been reviewed by Sinkar *et al.* (*J. Biosci. (Bangalore) 11*:47-58 (1987)).

[0134] Another preferred host is an insect cell, for example the *Drosophila* larvae. Using insect cells as hosts, the *Drosophila* alcohol dehydrogenase promoter can be used. Rubin, *Science 240*:1453-1459 (1988). In the alternative, baculovirus vectors can be engineered to express large amounts of PS1 in insects cells (Jasny, *Science 238*:1653 (1987); Miller *et al.*, In: *Genetic Engineering* (1986), Setlow, J.K., *et al.*, eds., *Plenum*, Vol. 8, pp. 277-297); Atkinson *et al.* (*Pestic. Sci. 28*:215-224 (1990)).

[0135] The PS1 gene may also be expressed in non-human transgenic animals such as mice, rats, rabbits, sheep, dogs and pigs (see Hammer *et al.*, *Nature 315*:680-683 (1985); Palmiter *et al.*, *Science 222*:809-814 (1983); Brinster *et al. Proc. Natl. Acad. Sci. USA* 82:4438-4442 (1985); Palmiter & Brinster, *Cell 41*:343-345 (1985); and U.S. Patent Nos. 5,175,383, 5,087,571, 4,736,866, 5,387,742, 5,347,075, 5,221,778, and 5,175,384). Briefly, an expression vector, including a nucleic acid molecule to be expressed together with appropriately positioned expression control sequences, is introduced into pronuclei of fertilized eggs, for example, by microinjection. Integration of the injected DNA is detected by blot analysis of DNA from tissue samples. It is preferred that the introduced DNA be incorporated into the germ line of the animal so that it is passed on to the animal's progeny. Tissue-specific expression may be achieved through the use of a tissue-specific promoter, or through the use of an inducible promoter, such as the metallothionein gene promoter (Palmiter *et al.*, *Science 222*:809-814 (1983)) which allows regulated expression of the transgene.

C. Protein Isolation

[0136] Proteins can be isolated by, among other methods, culturing suitable host and vector systems to produce the recombinant translation products of the present invention. Supernatants from such cell lines, or protein inclusions or whole cells

where the protein is not excreted into the supernatant, can then be treated by a variety of purification procedures in order to isolate the desired proteins. For example, the supernatant may be first concentrated using commercially available protein concentration filters. Then, the concentrate may be applied to a suitable purification matrix such as, for example, an anti-protein antibody bound to a suitable support. Alternatively, anion or cation exchange resins may be employed in order to purify the protein. As a further alternative, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps may be employed to further purify the protein. Other methods of isolating the proteins of the present invention are well known in the skill of the art.

[0137] A protein is deemed to be "isolated" within the context of the present invention if no other (undesired) protein is detected pursuant to SDS-PAGE analysis followed by Coomassie blue staining. Within other embodiments, the desired protein can be isolated such that no other (undesired) protein is detected pursuant to SDS-PAGE analysis followed by silver staining.

IV. Antibodies

[0138] Antibodies to the PS1 proteins may readily be prepared given the disclosure provided herein. Within the context of the present invention, antibodies are understood to include monoclonal antibodies, polyclonal antibodies, anti-ideotropic antibodies, antibody fragments (*e.g.*, Fab, and $F(ab')_2$, F_V variable regions, or complementarity determining regions). As discussed above, antibodies are understood to be specific against an Alzheimer disease protein if it binds with a K_a of greater than or equal to 10^{-7} M, preferably greater than or equal to 10^{-8} M. The affinity of a monoclonal antibody or binding partner can be readily determined by one of ordinary skill in the art.

[0139] Briefly, polyclonal antibodies may be readily generated by one of ordinary skill in the art from a variety of warm-blooded animals such as horses, cows, various fowl, rabbits, mice, or rats. Typically, a PS1 protein or unique PS1

peptide of 13-20 amino acids (preferably conjugated to keyhole limpet hemocyanin by cross-linking with glutaraldehyde) is utilized to immunize the animal through intraperitoneal, intramuscular, intraocular, or subcutaneous injections, an adjuvant such as Freund's complete or incomplete adjuvant. Following several booster immunizations, samples of serum are collected and tested for reactivity to the PS1 protein. Particularly preferred polyclonal antisera will give a signal on one of these assays that is at least three times greater than background. Once the titer of the animal has reached a plateau in terms of its reactivity to the protein, larger quantities of antisera may be readily obtained either by weekly bleedings, or by exsanguinating the animal.

[0140] Monoclonal antibodies may also be readily generated using conventional techniques (see U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993 which are incorporated herein by reference; *see also*, *Antibodies: A Laboratory Manual*, Harlow & Lane, eds., Cold Spring Harbor Press, Cold Spring Harbor, NY (1988), also incorporated herein by reference).

[0141] Briefly, within one embodiment a subject animal such as a rat or mouse is injected with a PS1 protein or portion thereof as described above. The protein may be admixed with an adjuvant such as Freund's complete or incomplete adjuvant in order to increase the resultant immune response. Between one and three weeks after the initial immunization the animal may be reimmunized with another booster immunization, and tested for reactivity to the protein utilizing assays described above. Once the animal has plateaued in its reactivity to the mutant, it is sacrificed, and organs which contain large numbers of B cells such as the spleen and lymph nodes are harvested.

[0142] Cells which are obtained from the immunized animal may be immortalized by transfection with a virus such as the Epstein-Barr virus (EBV) (*see*, Glasky & Reading, *Hybridoma 8*(4):377-389 (1989)). Alternatively, within a preferred embodiment, the harvested spleen and/or lymph node cell suspensions are fused with a suitable myeloma cell in order to create a "hybridoma" which secretes

monoclonal antibody. Suitable myeloma lines include, for example, NS-1 (ATCC No. TLB 18), an P3X63 - Ag 8.653 (ATCC No. CRL 1580).

[0143] Following the fusion, the cells may be placed into culture plates containing a suitable medium, such as RPMI 1640, or DMEM (Dulbecco's Modified Eagles Medium), as well as additional ingredients, such as fetal bovine serum. Additionally, the medium should contain a reagent which selectively allows for the growth of fused spleen and myeloma cells such as HAT (hypoxanthine, aminopterin, and thymidine) (Sigma Chemical Co., St. Louis, MO). After about seven days, the resulting fused cells or hybridomas may be screened in order to determine the presence of antibodies which are reactive against a PS1 protein. A wide variety of assays may be utilized to determine the presence of antibodies which are reactive against the proteins of the present invention, including for example, immunoelectrophoresis (IEP), radioimmunoassays, radioimmunoprecipitations, Enzyme-Linked Immuno-Sorbent Assays (ELISA), dot blot assays, western blots, immunoprecipitation, inhibition or competition Assays, and sandwich assays. Following several clonal dilutions and reassays, a hybridoma producing antibodies reactive against PS1 may be isolated.

[0144] Other techniques known in the art may also be utilized to construct monoclonal antibodies. In the alternative a commercial system available from Stratacyte, La Jolla, CA, enables the production of antibodies through recombinant techniques. Briefly, mRNA may be isolated from a B cell population, and utilized to create heavy and light chain immunoglobulin cDNA expression libraries in the λ ImmunoZap(H) and λ ImmunoZap(L) vectors. These vectors may be screened individually or co-expressed to form Fab fragments or antibodies. Positive plaques may subsequently be converted to a non-lytic plasmid which allows high level expression of monoclonal antibody fragments from *E. coli*.

[0145] Similarly, portions or fragments, such as Fab or Fv fragments, of antibodies may also be constructed utilizing conventional enzymatic digestion or recombinant DNA techniques to incorporate the variable regions of a gene which encodes a specifically binding antibody. In one embodiment of the present

invention, the genes which encode the variable region from a hybridoma producing a monoclonal antibody of interest are amplified using nucleotide primers for the variable region. These primers may be synthesized by one of ordinary skill in the art, or may be purchased from commercially available sources. Primers for mouse and human variable regions including, among others, primers for V_{Ha}, V_{Hb}, V_{Hc}, V_{Hd}, C_{H1}, V_L and C_L regions are available from, *e.g.*, Stratacyte (La Jolla, CA). The primers may be utilized to amplify heavy or light chain variable regions, which may then be inserted into vectors such as ImmunoZAP™ H or ImmunoZAP™ L (Stratacyte), respectively. These vectors may then be introduced into *E. coli* for expression. Utilizing these techniques, large amounts of a single-chain protein containing a fusion of the V_H and V_L domains may be produced (*see* Bird *et al.*, *Science 242*:423-426 (1988)). In addition, such techniques may be utilized to change a "murine" antibody to a "human" antibody, without altering the binding specificity of the antibody.

[0146] Once suitable antibodies have been obtained, they may be isolated or purified by many techniques well known to those of ordinary skill in the art (*see Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988). Suitable techniques include peptide or protein affinity columns, HPLC or RP-HPLC, purification on protein A or protein G columns, or any combination of these techniques.

[0147] Antibodies of the present invention have many uses. For example, antibodies may be utilized in flow cytometry to sort cells bearing such a PS1 protein. Briefly, in order to detect the protein or peptide of interest on cells, the cells are incubated with a labeled monoclonal antibody which specifically binds to the protein of interest, followed by detection of the presence of bound antibody. These steps may also be accomplished with additional steps such as washings to remove unbound antibody. Labels suitable for use within the present invention are well known in the art including, among others, fluorescein isothiocyanate (FITC), phycoerythrin (PE), horse radish peroxidase (HRP), and colloidal gold.

Particularly preferred for use in flow cytometry is FITC, which may be conjugated to purified antibody according to known methods.

[0148] Of special interest to the present invention are antibodies to PS1 which are produced in humans, or are "humanized" (*i.e.*, non-immunogenic in a human) by recombinant or other technology. Humanized antibodies may be produced, for example by replacing an immunogenic portion of an antibody with a corresponding, but non-immunogenic portion (*i.e.*, chimeric antibodies) (Robinson, R.R. *et al.*, PCT/US86/02269; Akira, K. *et al.*, EP-A 184,187; Taniguchi, M., EP-A 171,496; Morrison, S.L. *et al.*, EP-A 173,494; Neuberger, M.S. *et al.*, PCT Appl. WO 86/01533; Cabilly, S. *et al.*, EP-A 125,023; Better, M. *et al., Science 240*:1041-1043 (1988); Liu, A.Y. *et al., Proc. Natl. Acad. Sci. USA 84*:3439-3443 (1987); Liu, A.Y. *et al., J. Immunol. 139*:3521-3526 (1987); Sun, L.K. *et al., Proc. Natl. Acad. Sci. USA 84*:214-218 (1987); Nishimura, Y. *et al., Canc. Res. 47*:999-1005 (1987); Wood, C.R. *et al., Nature 314*:446-449 (1985)); Shaw *et al., J. Natl.Cancer Inst. 80*:1553-1559 (1988). General reviews of "humanized" chimeric antibodies are provided by Morrison, S.L. (*Science, 229*:1202-1207 (1985)) and by Oi, V.T. *et al., BioTechniques 4*:214 (1986)). Suitable "humanized" antibodies can be alternatively produced by CDR or CEA substitution (Jones, P.T. *et al., Nature 321*:552-525 (1986); Verhoeyan *et al., Science 239*:1534 (1988); Beidler, C.B. *et al., J. Immunol. 141*:4053-4060 (1988)).

V. Methods of Detecting The Presence of PS1 in a Sample

[0149] Assays useful within the context of the present invention include those assays for detecting agonists or antagonists of PS1 protein activity. Other assays are useful for the screening of peptide or organic molecule libraries. Still other assays are useful for the identification and/or isolation of nucleic acid molecules and/or peptides within the present invention, or for diagnosis of a patient with an increased likelihood of contracting Alzheimer's disease.

A. Nucleic Acid Based Diagnostic Tests

[0150] Briefly, the present invention provides probes and primers for detecting the PS1 genes and/or mutants thereof. For example, probes are provided that are capable of specifically hybridizing to PS1 genes, DNA or RNA. For purposes of the present invention, probes are "capable of hybridizing" to PS1 genes, DNA or RNA if they hybridize to a PS1 gene under conditions of either high or moderate stringency (see Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, *supra*), but typically not to the PS2 gene. Preferably, high stringency conditions would be used, such as 5x SSPE, 1x Denhardt's solution (Sambrook *et al.*, *supra*), 0.1% SDS at 65°C and at least one wash to remove excess probe in the presence of 0.2x SSC, 1x Denhardt's solution, 0.1% SDS at 65°C. Except as otherwise provided herein, probe sequences are designed to allow hybridization to PS1 genes, but not to DNA or RNA sequences from other genes. The probes are used, for example, to hybridize to nucleic acid that is present in a biological sample isolated from a patient. The hybridized probe is then detected, thereby indicating the presence of the desired cellular nucleic acid. Preferably, the cellular nucleic acid is subjected to an amplification procedure, such as PCR, prior to hybridization. Alternatively, the PS1 gene may be amplified, and the amplified product subjected to DNA sequencing.

[0151] Mutants of PS1 may be detected by DNA sequence analysis or hybridization with allele-specific oligonucleotide probes under conditions and for time sufficient to allow hybridization to the specific allele. Typically, the hybridization buffer and wash will contain tetramethyl ammonium chloride or the like (see Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, *supra*).

[0152] Probes of the present invention may be composed of DNA, RNA, nucleic acid analogues (*e.g.*, peptide/nucleic acids), or any combination thereof. They may be as small as about 12 nucleotides in length, usually about 14 to 18 nucleotides in length, but may possibly be as large as the entire sequence of a PS1

gene. Selection of probe size is somewhat dependent upon the use of the probe, and is within the skill of the art.

[0153] Suitable probes can be constructed and labeled using techniques that are well known in the art. Shorter probes of, for example, 12 bases can be generated synthetically and labeled with ^{32}P using T_4 polynucleotide kinase. Longer probes of about 75 bases to less than 1.5 kb are preferably generated by, for example, PCR amplification in the presence of labeled precursors such as [α-^{32}P]dCTP, digoxigenin-dUTP, or biotin-dATP. Probes of more than 1.5 kb are generally most easily amplified by transfecting a cell with a plasmid containing the relevant probe, growing the transfected cell into large quantities, and purifying, the relevant sequence from the transfected cells. (*See* Sambrook *et al.*, *supra.*)

[0154] Probes can be labeled by a variety of markers, including for example, radioactive markers, fluorescent markers, enzymatic markers, and chromogenic markers. The use of ^{32}P is particularly preferred for marking or labeling a particular nucleic acid probe.

[0155] Illustrative examples of suitable enzyme labels include malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast-alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, and acetylcholine esterase.

[0156] Illustrative examples of suitable radioisotopic labels include ^{3}H, ^{111}In, ^{125}I, ^{131}I, ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{57}To, ^{58}Co, ^{59}Fe, ^{75}Se, ^{152}Eu, ^{90}Y, ^{67}Cu, ^{217}Ci, ^{211}At, ^{212}Pb, ^{47}Sc, ^{109}Pd, etc. ^{111}In is a preferred isotope where *in vivo* imaging is used since its avoids the problem of dehalogenation of the ^{125}I or ^{131}I-labeled monoclonal antibody by the liver. In addition, this radionucleotide has a more favorable gamma emission energy for imaging (Perkins *et al., Eur. J. Nucl. Med. 10*:296-301 (1985); Carasquillo *et al., J. Nucl. Med. 28*:281-287 (1987)). For example, ^{111}In coupled to monoclonal antibodies with 1-(P-isothiocyanato-benzyl)-DPTA has shown little uptake in non-tumorous tissues, particularly the

liver, and therefore enhances specificity of tumor localization (Esteban *et al., J. Nucl. Med. 28*:861-870 (1987)).

[0157] Illustrative examples of suitable non-radioactive isotopic labels include ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Tr, and ^{56}Fe.

[0158] Illustrative examples of suitable fluorescent labels include an ^{152}Eu label, a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an allophycocyanin label, an o-phthaldehyde label, and a fluorescamine label.

[0159] Illustrative examples of suitable toxin labels include diphtheria toxin, ricin, and cholera toxin.

[0160] Illustrative examples of chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, and an aequorin label.

[0161] Illustrative examples of nuclear magnetic resonance contrasting agents include heavy metal nuclei such as Gd, Mn, and iron.

[0162] Typical techniques for binding the above-described labels to antibodies are provided by Kennedy *et al.*, *Clin. Chim. Acta 70*:1-31 (1976), and Schurs *et al.*, *Clin. Chim. Acta 81*:1-40 (1977). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method, all of which methods are incorporated by reference herein.

[0163] The probes of the present invention can be utilized to detect the presence of PS1 mRNA or DNA within a sample. However, if the nucleic acid is present in only a limited amount, then it may be beneficial to amplify the relevant sequence such that it may be more readily detected or obtained.

[0164] In the alternative, mutations can be visualized by single strand conformation polymorphism (SSCP) analysis.

[0165] A variety of methods may be utilized in order to amplify a selected sequence, including, for example, RNA amplification (see Lizardi *et al.*,

Bio/Technology 6:1197-1202 (1988); Kramer *et al.*, *Nature 339*:401-402 (1989); Lomeli *et al.*, *Clinical Chem. 35(9)*:1826-1831 (1989); U.S. Patent No. 4,786,600), and DNA amplification utilizing LCR or polymerase chain reaction ("PCR") (*see*, U.S. Patent Nos. 4,683,195, 4,683,202, and 4,800,159) (*see also* U.S. Patent Nos. 4,876,187 and 5,011,769, which describe an alternative detection/amplification system comprising the use of scissile linkages), or other nucleic acid amplification procedures that are well within the level of ordinary skill in the art.

[0166] With respect to PCR, for example, the method may be modified as known in the art, *e.g.*, *PCR Protocols, A Guide to Methods and Applications*, edited by Michael *et al.*, Academic Press, 1990, utilizing the appropriate chromosomal or cDNA library to obtain the fragment of the present invention. Transcriptional enhancement of PCR may be accomplished by incorporation of bacteriophage T7 RNA polymerase promoter sequences in one of the primary oligonucleotides, and immunoenzymatic detection of the products from the enhanced emitter may be effected using anti-RNA:DNA antibodies (Blais, *Appl. Environ. Microbiol. 60*:4348-352 (1994)). PCR may also be used in combination with reverse dot-blot hybridization (Iida *et al.*, *FEMS Microbiol. Lett. 114*:167-172 (1993)). PCR products may be quantitatively analyzed by incorporation of dUTP (Duplàa *et al.*, *Anal. Biochem. 212*:229-236 (1993)), and samples may be filter sampled for PCR-gene probe detection (Bej *et al.*, *Appl. Environ. Microbiol. 57*:3529-3534 (1991)).

[0167] In a particularly preferred embodiment, PCR amplification is utilized to detect PS1 gene DNA. Briefly, as described in greater detail below, a DNA sample is denatured at 95°C in order to generate single-stranded DNA. Specific primers are then annealed to the single-stranded DNA at 37°C to 70°C, depending on the proportion of AT/GC in the primers. The primers are extended at 72°C with *Taq* DNA polymerase in order to generate the opposite strand to the template. These steps constitute one cycle, which may be repeated in order to amplify the selected sequence.

[0168] In an alternative preferred embodiment, LCR amplification is utilized for amplification. LCR primers are synthesized such that the 5′ base of the upstream primer is capable of hybridizing to a unique base pair in a desired gene to specifically detect a PS1 gene. While in another preferred embodiment, the probes are used in an automated, non-isotopic strategy wherein target nucleic acid sequences are amplified by PCR, and then desired products are determined by a calorimetric oligonucleotide ligation assay (OLA) (Nickerson *et al.*, *Proc. Natl. Acad. Sci. USA 81*:8923-8927 (1990)).

[0169] Primers for the amplification of a selected sequence should be selected from sequences that are highly specific and form stable duplexes with the target sequence. The primers should also be non-complementary, especially at the 3′ end, should not form dimers with themselves or other primers, and should not form secondary structures or duplexes with other regions of DNA. In general, primers of about 18 to 20 nucleotides are preferred, and can be easily synthesized using techniques well known in the art. PCR products, and other nucleic acid amplification products, may be quantified using techniques known in the art, *i.e.*, SSCP analysis.

B. Diagnostic Kits Comprising Nucleic Acid Probes to PS1

[0170] In another embodiment, the present invention relates to a kit for detecting the presence of PS1 in a sample comprising at least one container means having disposed therein the above-described nucleic acid probe. In a preferred embodiment, the kit further comprises other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound nucleic acid probe. Examples of detection reagents include, but are not limited to radiolabelled probes, enzymatic labeled probes (horse radish peroxidase, alkaline phosphatase), and affinity labeled probes (biotin, avidin, or steptavidin).

[0171] In detail, a compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers,

plastic containers or strips of plastic or paper. Such containers allow the efficient transfer of reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the probe or primers used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, and the like), and containers which contain the reagents used to detect the hybridized probe, bound antibody, amplified product, or the like.

[0172] Types of detection reagents include labeled secondary probes, or in the alternative, if the primary probe is labeled, the enzymatic, or antibody binding reagents which are capable of reacting with the labeled probe. One skilled in the art will readily recognize that the disclosed probes and amplification primers of the present invention can readily be incorporated into one of the established kit formats which are well known in the art.

C. Antibody-based Diagnostic Tests and Kits

[0173] The present invention further provides antibodies, as discussed above, for the detection of PS1 gene products in diagnostic tests and kits. A variety of assays can be utilized in order to detect antibodies that specifically bind to the desired protein or peptide. Exemplary assays are described in detail in *Antibodies: A Laboratory Manual*, Harlow and Lane, *supra*. Representative examples of such assays include IEP, radioimmunoassays, radioimmunoprecipitations, ELISA, dot blot assays, inhibition or competition assays, and sandwich assays, immunostick (dipstick) assays, simultaneous immunoassays, immunochromatographic assays, immunofiltration assays, latex bead agglutination assays, immunofluorescent assays, biosensor assays, and low-light detection assays, and the like (*see, e.g., Antibodies: A Laboratory Manual*, *supra*).

[0174] A fluorescent antibody test (FA-test) uses a fluorescently-labeled antibody able to bind to one of the proteins of the invention. Visual determinations using fluorescence microscopy yield a qualitative result. In a preferred embodiment, this assay is used for the examination of tissue samples and histological sections.

[0175] In latex bead agglutination assays, antibodies to one or more of the proteins of the present invention are conjugated to latex beads. The antibodies conjugated to the latex beads are then contacted with a sample under conditions permitting antibodies to bind to desired proteins in the sample, if any. Visual results yield a qualitative result. This method is preferred in the field for on-site testing.

[0176] Enzyme immunoassays (EIA) include a number of different assays able to utilize the antibodies provided by the present invention. For example, a heterogeneous indirect EIA uses a solid phase coupled with an antibody of the invention and an affinity purified, anti-IGg immunoglobulin preparation. Preferably, the solid phase is a polystyrene microtiter plate. The antibodies and immunoglobulin preparation are then contacted with the sample under conditions permitting antibody binding, which conditions are well known in the art. The results of such an assay can be read visually, but are preferably read using a spectrophotometer, such as an ELISA plate reader, to yield a quantitative result.

[0177] An alternative solid phase EIA format includes a plastic-coated ferrous metal beads able to be moved during the procedures of the assay by means of a magnet. Yet another alternative is a low-light detection immunoassay format. In this highly sensitive format, the light emission produced by appropriately labeled bound antibodies are quantified automatically, preferably, using microtiter plates.

[0178] In a capture-antibody sandwich enzyme assay, the desired protein is bound between an antibody attached to a solid phase, preferably a polystyrene microtiter plate, and a labeled antibody. Preferably, the results are measured using a spectrophotometer, such as an ELISA plate reader. In an alternative embodiment, a radioactive tracer is substituted for the enzyme mediated detection in an EIA to produce a radioimmunoassay (RIA).

[0179] In a sequential assay format, reagents are allowed to incubate with the capture antibody in a step wise fashion. The test sample is first incubated with the capture antibody. Following a wash step, an incubation with the labeled antibody occurs. In a simultaneous assay, the two incubation periods described in the sequential assay are combined. This eliminates one incubation period plus a wash step.

[0180] A dipstick/immunostick format is essentially an immunoassay except that the solid phase, instead of being a polystyrene microtiter plate, is a polystyrene paddle or dipstick. Reagents are the same and the format can either be simultaneous or sequential.

[0181] In a chromatographic strip test format, a capture antibody and a labeled antibody are dried onto a chromatographic strip, which is typically nitrocellulose or nylon of high porosity bonded to cellulose acetate. The capture antibody is usually spray dried as a line at one end of the strip. At this end there is an absorbent material that is in contact with the strip. At the other end of the strip the labeled antibody is deposited in a manner that prevents it from being absorbed into the membrane. Usually, the label attached to the antibody is a latex bead or colloidal gold. The assay may be initiated by applying the sample immediately in front of the labeled antibody.

[0182] Immunofiltration/immunoconcentration formats combine a large solid phase surface with directional flow of sample/reagents, which concentrates and accelerates the binding of antigen to antibody. In a preferred format, the test sample is preincubated with a labeled antibody then applied to a solid phase such as fiber filters or nitrocellulose membranes or the like. The solid phase can also be precoated with latex or glass beads coated with capture antibody followed by detection by standard immunoassay techniques. The flow of sample/reagents can be modulated by either vacuum or the wicking action of an underlying absorbent material.

[0183] A threshold biosensor assay is a sensitive, instrumented assay amenable to screening large number of samples at low cost. In one embodiment, such an assay

comprises the use of light addressable potentiometric sensors wherein the reaction involves the detection of a pH change due to binding of the desired protein by capture antibodies, bridging antibodies and urease-conjugated antibodies. Upon binding, a pH change is effected that is measurable by translation into electrical potential (μvolts). The assay typically occurs in a very small reaction volume, and is very sensitive. Moreover, the reported detection limit of the assay is 1,000 molecules of urease per minute.

[0184] One type of test sample which can be utilized in the present invention is derived from amniotic fluid or cells. Such a test sample is utilized to identify fetuses which carry a human gene or mutation for FAD.

D. Diagnostic Kits Comprising Antibodies to PS1

[0185] In another embodiment of the present invention, a kit is provided which contains all the necessary reagents to carry out the previously described methods of detection. The kit may comprise: i) a first container means containing an above-described antibody, and ii) second container means containing a conjugate comprising a binding partner of the antibody and a label. In another preferred embodiment, the kit further comprises one or more other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound antibodies. Examples of detection reagents include, but are not limited to, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the chromophoric, enzymatic, or antibody binding reagents which are capable of reacting with the labeled antibody. One skilled in the art will readily recognize that the antibodies described in the present invention can readily be incorporated into one of the established kit formats which are well known in the art.

E. Anti-peptide Antibodies

[0186] In another embodiment, the peptide, in particular the PS1 peptide, is used to generate an antibody which is capable of binding to the peptide (e.g., anti-PS1 peptide antibodies). The anti-peptide antibodies of the present invention may include monoclonal and polyclonal antibodies, as well fragments of these antibodies, and humanized forms. Humanized forms of the antibodies of the present invention may be generated using one of the procedures known in the art such as chimerization or CDR grafting.

[0187] Moreover, the invention also provides hybridomas which are capable of producing the above-described antibodies.

[0188] Furthermore, one skilled in the art can readily adapt currently available procedures, as well as the techniques, methods and kits disclosed above with regard to antibodies, to generate peptides capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby *et al.*, "Application of Synthetic Peptides: Antisense Peptides", in *Synthetic Peptides, A User's Guide*, W.H. Freeman, NY, pp. 289-307 (1992), and Kaspczak *et al., Biochemistry 28:*9230-8 (1989).

[0189] Anti-peptide peptides can be generated in one of two fashions. First, the anti-peptide peptides can be generated by replacing the basic amino acid residues found in the peptide sequence, e.g., the IT-11 peptide sequence, with acidic residues, while maintaining hydrophobic and uncharged polar groups. For example, lysine, arginine, and/or histidine residues are replaced with aspartic acid or glutamic acid and glutamic acid residues are replaced by lysine, arginine or histidine.

[0190] Alternatively, the anti-peptide peptides of the present invention can be generated by synthesizing and expressing a peptide encoded by the antisense strand of the DNA which encodes the peptides, preferably the IT-11 peptide. Peptides produced in this fashion are, in general, similar to those described above

since codons complementary to those coding for basic residues generally code for acidic residues.

[0191] One skilled in the art will readily recognize that the antibodies described in the present invention can readily be incorporated into one of the established kit formats which are well known in the art.

F. Other Assays

[0192] Transmembrane receptors are involved in many cellular communication process and have been the targets of numerous pharmacologic screening assays for the identification and development of new therapeutic agents. Many of these screening assays look for ligand induced changes in cell lines expressing the recombinant receptor. In some cases second messengers are assayed directly while in others, receptor is transfected into a cell line carrying a reporter gene construct whose expression level can be influenced (positively or negatively) by functional activation of the receptor. One common result of the stimulation of many different second messenger systems is transient changes in intracellular calcium homeostasis. This can be the result of Ca^{2+} release from various intracellular compartments or from the influx of extracellular calcium.

[0193] Calcium transients offer a highly sensitive and selective method for characterization of PS1 gene function. Expression of recombinant PS1 in cell lines previously transfected with an aequorin reporter construct can be used to screen for and identify a PS1 ligand. Aequorin is a 21 kDa photoprotein that upon Ca^{2+} binding undergoes an irreversible reaction with the production of light in the visible range. Because the fractional rate of aequorin consumption is proportional in the physiological $[Ca^{2+}]$, it has been used for many years as a sensitive indicator of intracellular calcium. More recently, several different aequorin cDNA's have been engineered which allow selective targeting of aequorin expression to different intracellular compartments, including the cytoplasm, the nucleus and the endoplasmic reticulum. This allows for a variety of second messenger coupled

pathways/compartments to be screened. Identification of the PS1 ligand and determination of its signaling pathway will be a first step in the functional characterization of the PS1 gene. A cell line expressing mutant PS1 can be set up and screened in parallel in order to identify compounds which modify the mutant protein function in a way that mimics wild-type PS1 activity.

VI. Methods of Treating or Preventing Alzheimer's Disease

[0194] The present invention also provides methods for treating, or preventing Alzheimer disease, comprising the step of administering to a patient a vector (*e.g.*, expression vector, viral vector, or viral particle containing a vector), as described above, thereby reducing, the likelihood or delaying the onset of Alzheimer's disease.

[0195] Similarly, therapeutic peptides, peptidomimetics, or small molecules may be used to delay onset of Alzheimer's disease, lessen symptoms, or halt or delay progression of the disease. Such therapeutics may be tested in a transgenic animal model that expresses mutant protein, wild-type and mutant protein, or in an *in vitro* assay system.

[0196] One such *in vitro* assay system measures the amount of amyloid protein produced. Briefly, by way of illustration, a cell expressing both PS1 gene product and amyloid is cultured in the presence of a candidate therapeutic molecule. The PS1 protein expressed by the cell may be either wild-type or mutant protein. In either case, the amount of amyloid protein that is produced is measured from cells incubated with or without (control) the candidate therapeutic. Briefly, by way of example, cells are labeled in medium containing ^{35}S-methionine and incubated in the presence (or absence) of candidate therapeutic. Amyloid protein is detected in the culture supernatant by immunoprecipitation and SDS-PAGE electrophoresis or by ELISA. A statistically significant reduction of amyloid protein compared to the control signifies a therapeutic suitable for use in preventing or treating Alzheimer's disease.

[0197] Alternatively, transgenic animals expressing Alzheimer's disease protein may be used to test candidate therapeutics. Amyloid protein is measured or, if the animals exhibit other disease symptoms, such as memory or learning deprivation, an increase in memory or learning is measured. Memory and learning are tested in rodents by the Morris water maze (Stewart and Morris in *Behavioral Neuroscience*, R. Saghal Ed. (IRLPress, 1993, p. 107) and the Y-maze (Brits *et al.*, *Brain Res. Bull. 6*:71 (1981)). Therapeutics are administered to animals prior to testing. The response time in trials are measured and an improvement in memory and learning is demonstrated by a statistically significant decrease in the timed trials.

[0198] As noted above, the present invention provides methods for treating or preventing Alzheimer's disease through the administration to a patient of a therapeutically effective amount of an antagonist or pharmaceutical composition as described herein. Such patients may be identified through clinical diagnosis based on symptoms of dementia or learning and memory loss which are not attributable to other causes. In addition, patients are also identified through diagnosis of brain atrophy as determined by magnetic resonance imaging.

[0199] In another embodiment of the present invention, methods are presented for decreasing the expression of the PS1 peptide disclosed herein. Specifically, anti-sense RNA expression is used to disrupt the translation of the genetic message. In detail, a cell is modified using routine procedures such that it expresses an antisense message, a message which is complementary to the PS1 message. By constitutively or inducibly expressing the antisense RNA, the translation of PS1 mRNA can be regulated.

[0200] Cognitive behavior in AD may be measured by any one of several tests (*See* Gershon *et al.*, *Clinical Evaluation of Psychotropic Drugs: Principles and Guidelines*, Prien and Robinson (eds.), Raven Press, Ltd., New York, 1994, p. 467). One such test, BCRS, is designed to measure only cognitive functions: concentration, recent memory, past memory, orientation, functioning, and self-care. This test, as well as the Weschler Memory Scale and the Alzheimer's

Disease-Associated Scale, may be used to determine improvement following therapeutic treatment. "Improvement" in Alzheimer's disease is present if there is a statistically significant difference in the direction of normality in the Weschier Memory Scale test. For example, test results of the performance of treated patients as are compared to members of the placebo group or between subsequent tests given to the same patient. Improvement within the present invention also encompasses a delay in the age of onset of Alzheimer's disease.

A. Pharmaceutical Compositions

[0201] The present invention also provides a variety of pharmaceutical compositions, comprising one of the PS1 proteins, nucleic acid molecules, vectors, antibodies, host cells, agonists or antagonists or diluents. Generally, such carriers should be nontoxic to recipients at the dosages and concentrations employed. Ordinarily, the preparation of such composition entails combining the therapeutic agent with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 amino acid residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with nonspecific serum albumin are exemplary appropriate diluents.

[0202] In addition, the pharmaceutical compositions of the present invention may be prepared for administration by a variety of different routes, although intracranial routes are typically preferred. In addition, pharmaceutical compositions of the present invention may be placed within containers, along with packaging material which provides instructions regarding the use of such pharmaceutical compositions. Generally, such instructions will include a tangible expression describing the reagent concentration, as well as within certain embodiments, relative amounts of excipient ingredients or diluents (*e.g.*, water,

saline or PBS) which may be necessary to reconstitute the pharmaceutical composition.

[0203] As will be evident to one of skill in the art, the amount and frequency of administration will depend, of course, on such factors as the nature and severity of the indication being treated, the desired response, the condition of the patient, and so forth. Typically, the compositions may be administered by a variety of techniques, although intra-cranial routes are often preferred.

[0204] More specifically, the pharmaceutical compositions of the present invention will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient, the site of delivery of the polypeptide composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" of pharmaceutical composition for purposes herein is thus determined by such considerations.

[0205] As a general proposition, the total pharmaceutically effective amount of active ingredient administered parenterally per dose will be in the range of about 1 μg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the composition is typically administered at a dose rate of about 1 μg/kg/hour to about 50 μg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The key factor in selecting an appropriate dose is the result obtained. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

[0206] Pharmaceutical compositions containing the PS1 proteins, nucleic acid molecules, vectors, antibodies, host cells, agonists or antagonists of the invention may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch),

bucally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

[0207] The pharmaceutical composition is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. *et al., Biopolymers 22*:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (R. Langer *et al., J. Biomed. Mater. Res. 15*:167-277 (1981), and R. Langer, *Chem. Tech. 12*:98-105 (1982)), ethylene vinyl acetate (R. Langer *et al., Id.*) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomally entrapped PS1 proteins, nucleic acid molecules, vectors, antibodies, host cells, agonists or antagonists. Such liposomes are prepared by methods known *per se*: DE 3,218,121; Epstein *et al., Proc. Natl. Acad. Sci. (USA) 82*:3688-3692 (1985); Hwang *et al., Proc. Natl. Acad. Sci. (USA) 77*:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimaltherapy.

[0208] For parenteral administration, in one embodiment, the composition is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the

formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

[0209] Generally, the formulations are prepared by contacting the PS1 proteins, nucleic acid molecules, vectors, antibodies, host cells, agonists or antagonists uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

[0210] The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

[0211] The PS1 proteins, nucleic acid molecules, vectors, antibodies, host cells, agonists or antagonists are generally formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

[0212] Pharmaceutical compositions to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic compositions

generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0213] Pharmaceutical compositions ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous PS1 protein solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized PS1 polypeptide using bacteriostatic Water-for-Injection.

[0214] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

[0215] Within other embodiments of the invention, the vectors which contain or express the nucleic acid molecules which encode a PS1 protein, or even the nucleic acid molecule *per se* may be administered by a variety of alternative techniques, including for example administration of asialoosomucoid (ASOR) conjugated with poly (L-lysine) DNA complexes (Cristano *et al.*, *Proc. Natl. Acad. Sci. USA* 92122-92126 (1993)), DNA linked to killed adenovirus (Curiel *et al.*, *Hum. Gene Ther. 3(2)*:147-154 (1992)), cytofectin-mediated introduction (DMRIE-DOPE, Vical, Calif.), direct DNA injection (Acsadi *et al.*, *Nature 352*:815-818 (1991)); DNA ligand (Wu *et al.*, *J. Biol. Chem. 264*:16985-16987, 1989); lipofection (Felgner *et al.*, *Proc. Natl. Acad. Sci. USA 84*:7413-7417 (1989)); liposomes (Pickering *et al.*, *Circ. 89(1)*:13-21 (1994); and Wang *et al.*, *Proc. Natl. Acad. Sci. USA 84*:7851-7855 (1987)); microprojectile bombardment

(Williams *et al.*, *Proc. Natl. Acad. Sci. USA 88*:2726-2730 (1991)); and direct delivery of nucleic acids which encode the PS1 protein alone (Vile and Hart, *Cancer Res. 53*:3860-3864 (1993)), or utilizing PEG-nucleic acid complexes.

[0216] All patents and publications mentioned hereinabove are hereby expressly incorporated in their entirety by reference.

[0217] In order that those skilled in the art can more fully understand this invention the following examples are set forth. These examples are given solely for the purpose of illustration, and should not be considered as expressing limitations unless so set forth in the appended claims.

EXAMPLES

[0218] In the following examples and protocols, restriction enzymes, ligase, and all commercially available reagents were utilized in accordance with the manufacturer's recommendations. Standard methods and techniques for cloning and molecular analysis, as well as the preparation of standard reagents were performed essentially in accordance with *Molecular Cloning: A Laboratory Manual, second edition*, edited by Sambrook, Fritsch & Maniatis, Cold Spring Harbor Laboratory, 1989).

EXAMPLE 1
Screening of S182 Gene

[0219] Although early-onset AD is less common than late-onset AD, the PS1 locus is associated with the most aggressive form of the disease (onset 30-60 years), suggesting the importance of mutations in the PS1 locus with regard to causative effects of AD. The PS1 locus has been isolated to the region between D14S53 and D15S58 on human chromosome 14. Within that region, Sherrington *et al.*, *Nature 375*:754-760 (1995) reported the cloning of a novel gene, S182,

with five missense mutations in seven pedigrees segregating early-onset autosomal dominant AD.

[0220] To confirm the nucleotide sequence differences, and to assess the segregation in each FAD pedigree and the frequency in the general population (age >65 years), the exon of S182 containing the L286V mutation reported by Sherrington *et al., supra*, was screened as follows. First the PCR amplified exon containing the L286V mutation was restriction digested by *Pvu* II as described by Sherrington *et al., supra*; then the fragments were analyzed by means of a single strand conformation polymorphism (SSCP) analysis. The analysis was performed on 29 early-onset FAD kindreds (who are also negative for the five Sherrington *et al.*-reported mutations in S182) and from the 12 late-onset families. Each kindred was represented by two patients in the analysis. Also included were samples from 53 age matched controls from the FAD kindred to ascertain the validity of the results.

[0221] Genomic DNA from peripheral blood samples was PCR amplified to expedite the screening process. The PCR mixture was prepared for each sample according to the following protocol. Each 10 μL PCR reaction mixture contained: 1 μL *Taq*® buffer; 1.25 mM dNTPs (10 μL d[A,T,G,C] TP each stock, 760 μL HPLC water); 1 μL diluted primer mix (8 μL primers 7672* and 7673* in 200 μ HPLC water); 0.1 μL *Taq*®DNA polymerase; 0.1 μL α ^{32}P-dATP; 1 μL 1:50 genomic DNA (approx. 40 ng, diluted with HPLC water); 5.2 μL HPLC water. The reaction conditions for each 10 μl reaction volume were: 94°C for 4 min.; followed by 30 cycles of (94°C for 1 min.; 58°C for 1 min.; 72°C for 1 min.); then 72°C for 10 min.; followed by soaking at 4°C until removed and stored at -20°C.

[0222] * Primer KM 7672 Sequence: CACCCATTTACAAGTTTAGC (SEQ ID NO:5);

[0223] * Primer KM 7673 Sequence: GATGAGACAAGTGCCGTGAA (SEQ ID NO:6).

[0224] After amplification, 3 μl of each PCR reaction mixture was removed from under the oil and transferred to a new plate containing 30 μl of SSCP dilution mix

in each corresponding well. (SSCP dilution mix: 250 µl 20% SDS,; 1 ml 0.5M EDTA; Millipore® water to 50 ml). 30 µl 95% formamide dye mix was then added to each SSCP-diluted 33 µl sample. (Formamide dye mix: 0.25% bromophenol blue; 0.25% xylene cyanol FF; 95% formamide).

[0225] After 30 µl of the diluted, dyed sample was removed and set aside on ice for later use as a non-denaturing control, the samples were denatured at 90°C for 10 min., then placed on ice. The denatured samples were loaded onto a Mutation Detection Enhancement (MDE™) gel (FMC® Bioproducts, Rockland, ME) in 0.6X TBE buffer and run at 15 Watts for 20 hours. (MDE™ gel: 25% 2X gel concentrate; 10% glycerol; 0.6X TBE; to volume with HPLC water). Positive, negative and non-denatured controls were run with each gel, and a water control was run in one gel. The dyes permitted visualization and rapid comparison of the genetic mutations and polymorphisms in contrast to the normal (wild-type) samples.

[0226] Using the SSCA analysis, the sequence obtained from a patient carrying a mutation within the S182 exon can be potentially distinguished from that of a normal control individual. One or more mutation(s) in the S182 exon effecting a conformational change in the secondary/tertiary structure can be quickly visualized in the single stranded molecule. The MDE™ gel is designed to permit more compact molecules to run more quickly through the pores of the size differentiating gel, so that a mutated species is revealed as a band in the gel at a different point than that which is consistently seen in normal (control) samples encoded by the same S182 exon region.

[0227] The SSCP analysis did not identify the L286V mutation reported by Sherrington *et al., supra*; however, it did reveal three heterozygous nucleotide substitutions in PS1 in specific probands (*see* Example 2), which were not found in other pedigrees. Moreover, none of the three mutations was observed in the 106 chromosomes from age-matched controls used to ascertain the FAD pedigrees tested.

EXAMPLE 2

Detection of Mutations

[0228] Three previously unidentified, but apparently pathogenic, mutations in the S182 gene on chromosome 14 have been discovered that appear to cause early-onset forms of familial Alzheimer's disease (FAD). Specifically, the pathogenic mutations found in the S182 exon were: (1) T→C at nucleotide position 1035 (SEQ ID NO:27); (2) C→T at nucleotide position 1039 (SEQ ID NO:29); and (3) G→A at nucleotide position 1054 (SEQ ID NO:31). Each of the exonic mutations are missense substitutions which occur immediately at the C-terminal side of the sixth predicted transmembrane domain (TMD6) of the PS1 protein.

[0229] The first mutation results in an amino acid substitution at residue 263 of an arginine for a cysteine (C263R). The second mutation results in an amino acid substitution at residue 264 of a leucine for a proline (P264L). The third mutation results in an amino acid substitution at residue 269 of a histidine for an arginine (R269H).

[0230] In addition, two polymorphisms in the intronic sequence flanking the exon of S182 were found: (1) A->C, at nucleotide position -16 of the intron situated 3' of the exon; and (2) A->G at nucleotide position -20 of the intron situated 5' of the same exon.

[0231] C263R occurs in the proband of pedigree MGH12. At onset the proband was 47 years old. Autopsy results confirmed that the proband was afflicted with Alzheimer's disease. The C263R mutation was also found in all four other affected individuals from the same pedigree, MGH12 (average age at onset was 50 years).

[0232] P264L was observed in the proband of pedigree MGH6. At onset the proband was 45 years old, with a history of thyroid problems. The proband's brother developed AD at 50 years of age, and was autopsy-confirmed as having AD.

[0233] R269H was observed in a sporadic case of early-onset Alzheimer's disease. The patient's memory impairment began at about age 47, and he died at age 56. The neuropathology discovered during autopsy confirmed the earlier clinical diagnosis of AD, and the patient was found to have moderate congofilic angiopathy. The patient's father died in his early 60's of stroke, but he had presented a clinical picture of memory decline and progressive cognitive degeneration beginning in his mid-50's. The patient's grandfather (on his father's side) dies in his early 70's, but his previous history presented a gradual cognitive impairment that may have been ongoing since his mid-60's.

[0234] No formal clinical or neuropathological diagnosis was established for any member of this family exhibiting mutation R269H. At the time of the study, the patient's mother remained alive and well, without cognitive impairment; whereas his sister died of cancer in her late 50's-early 60's; the sister's son, however, remained healthy.

[0235] The fact that the newly identified mutations are presumably pathogenic is strongly supported by the profound effect that the substitutions impart on the resulting protein. The C263R, P264L, and R269H mutations reside in the predicted hydrophilic loop domain, and immediately follow the C-terminus of TMD6. Consequently, the mutations could extend the length of the transmembrane domain, thereby aberrantly affecting the anchorage of the protein in the membrane. Alternatively, the mutations may adversely affect the secondary/tertiary structure of the hydrophilic loop and/or the entire protein.

[0236] It is interesting to note that each of the newly identified mutations fall in the region in and around TMD6, which also contains the A246E mutation reported by Sherrington *et al., Nature* (1995), *supra*. Moreover, the average age at onset of AD in the three individuals or families characterized by the newly identified mutations is very similar (approximately age 50) to those having the A246E mutation. This indicates that disruptions in the PS1 protein, particularly in and around TMD6, may result in similar pathogenic consequences.

Accordingly, the newly identified mutations represent the most significant amino acid changes reported in S182 to date affecting early onset AD.